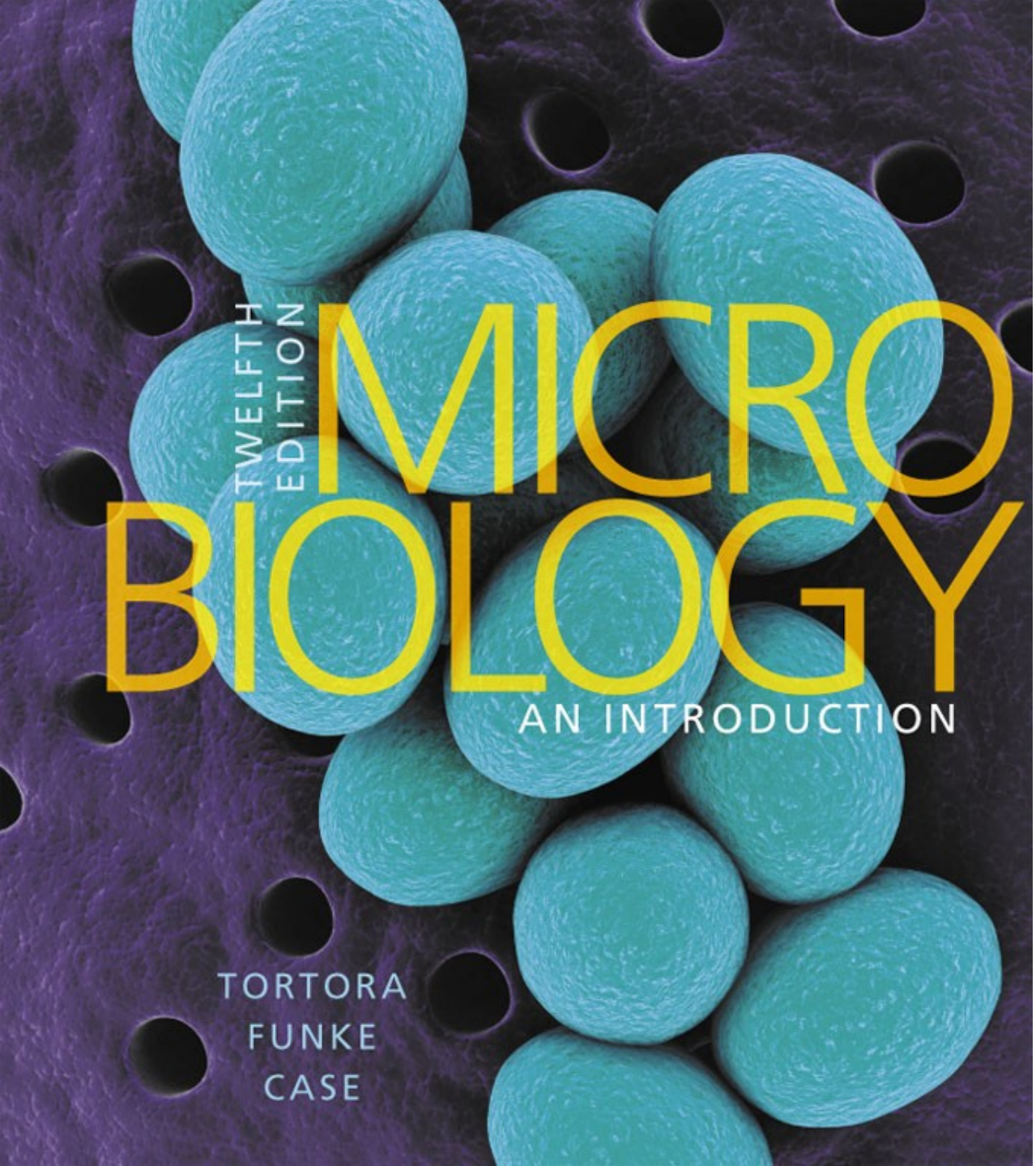
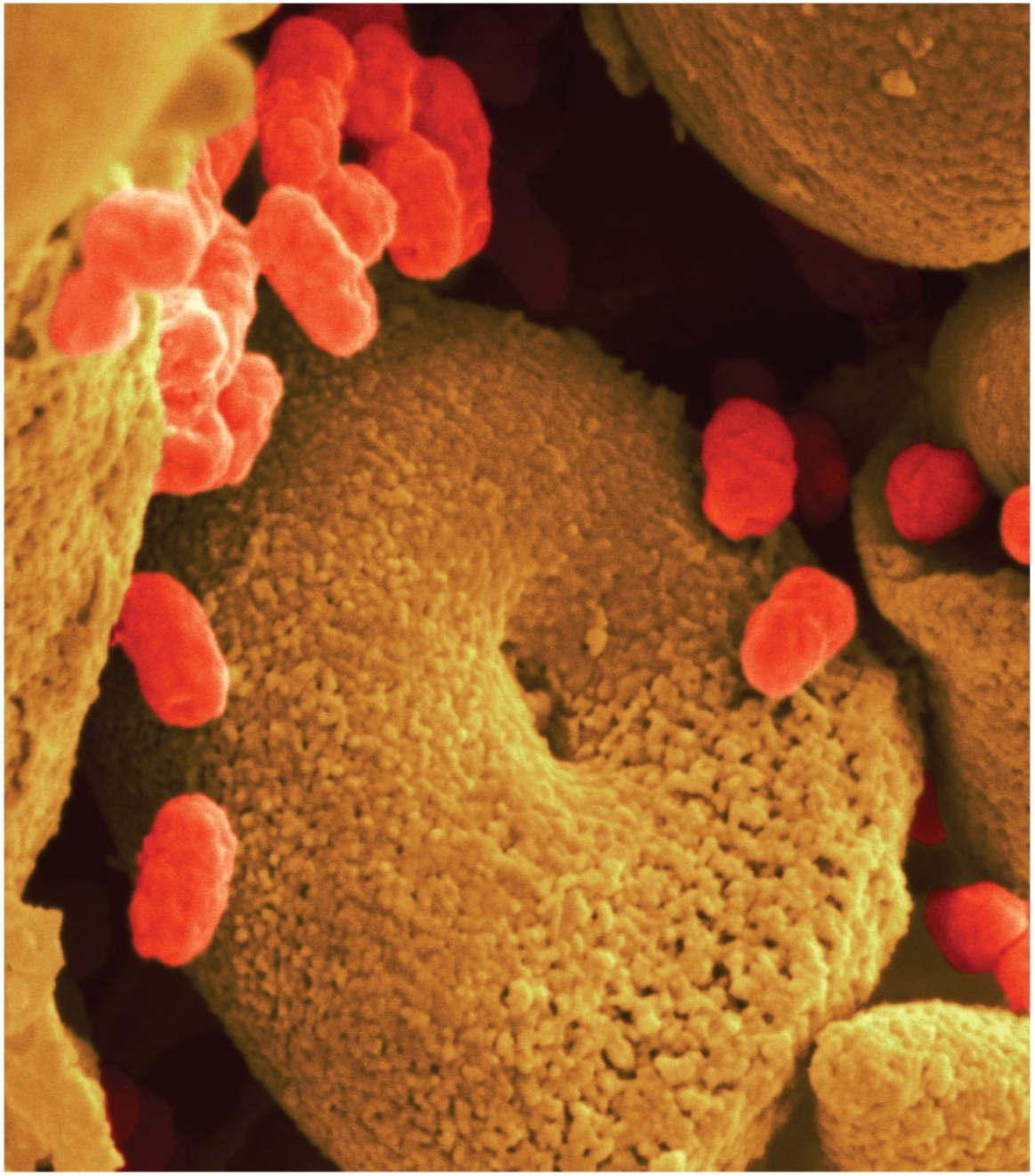


PowerPoint® Lecture Presentations prepared by
Bradley W. Christian, McLennan Community College

CHAPTER 6

Microbial Growth



The Requirements for Growth

Learning Objectives

6-1 Classify microbes into five groups on the basis of preferred temperature range.

6-2 Identify how and why the pH of culture media is controlled.

6-3 Explain the importance of osmotic pressure to microbial growth.

The Requirements for Growth

- Physical requirements
  - Temperature
  - pH
  - Osmotic pressure
- Chemical requirements
  - Carbon
  - Nitrogen, sulfur, and phosphorous
  - Trace elements
  - Oxygen
  - Organic growth factors

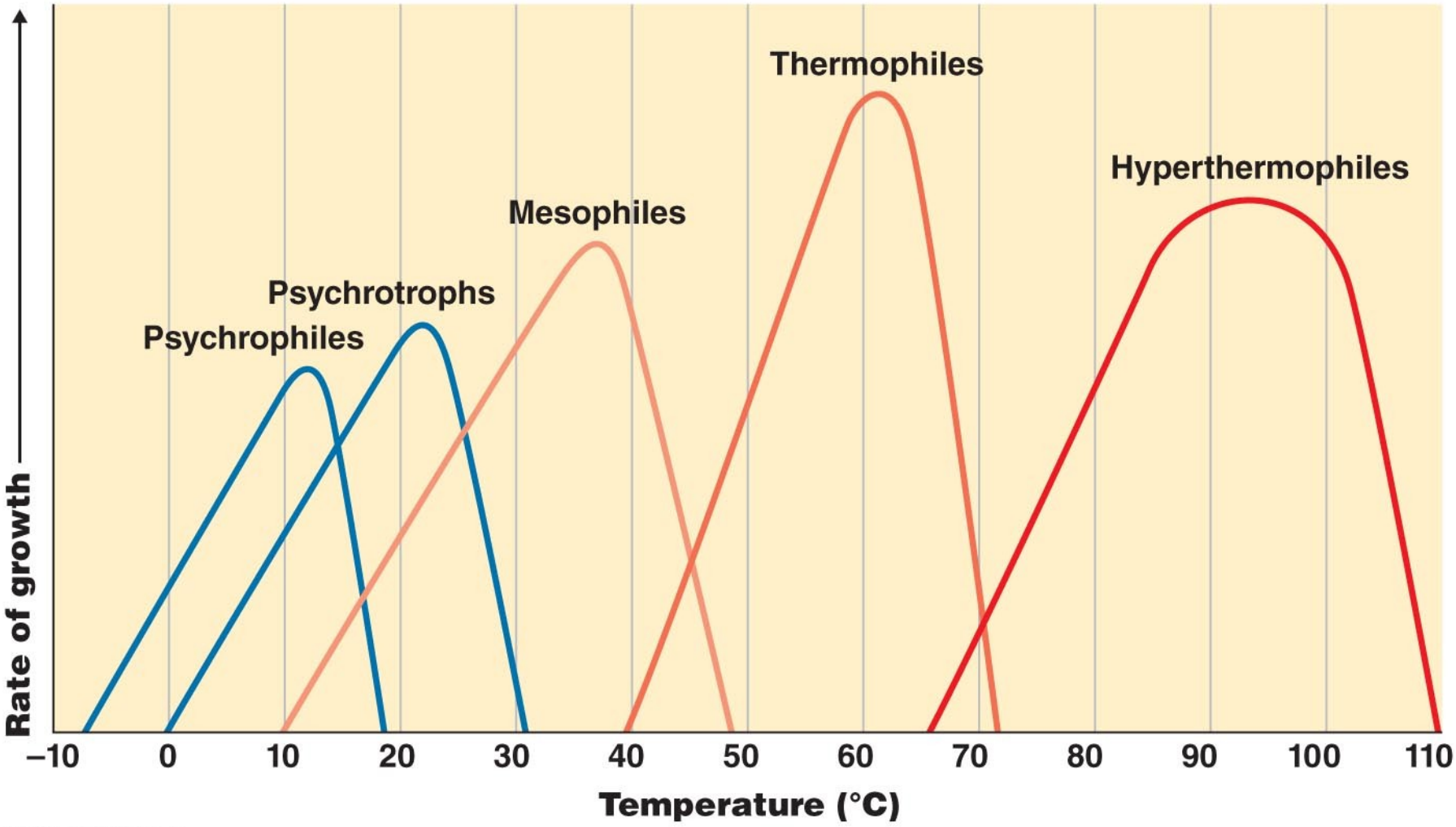
Physical Requirements

- Temperature
  - **Minimum growth temperature**
  - **Optimum growth temperature**
  - **Maximum growth temperature**

Physical Requirements

- Temperature (cont'd)
  - **Psychrophiles**—cold-loving
  - **Mesophiles**—moderate-temperature-loving
  - **Thermophiles**—heat-loving

Figure 6.1 Typical growth rates of different types of microorganisms in response to temperature.



Temperature

- **Psychrotrophs**
  - Grow between 0℃ and 20 to 30℃
  - Cause food spoilage

Figure 6.2 Food preservation temperatures.

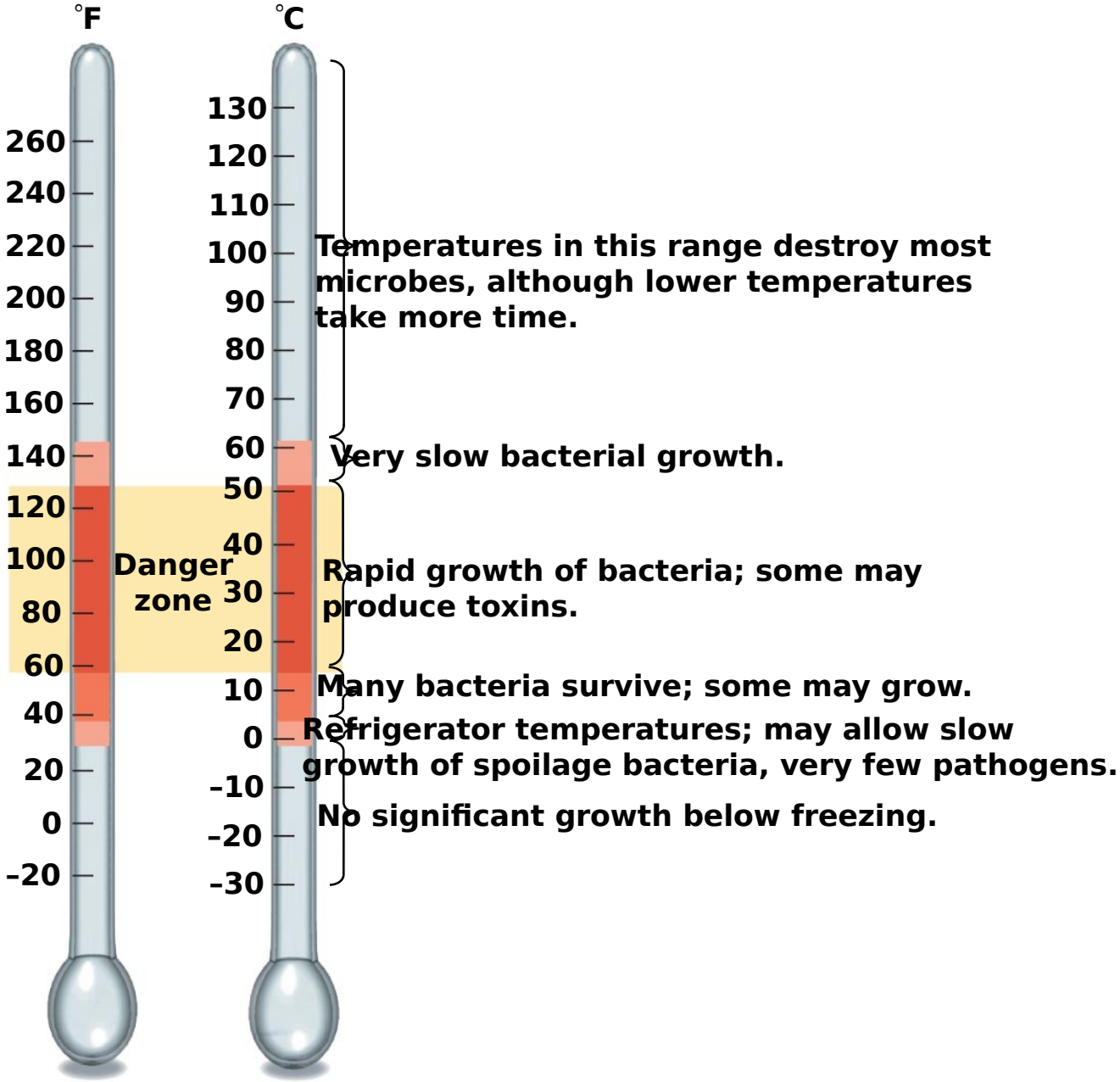
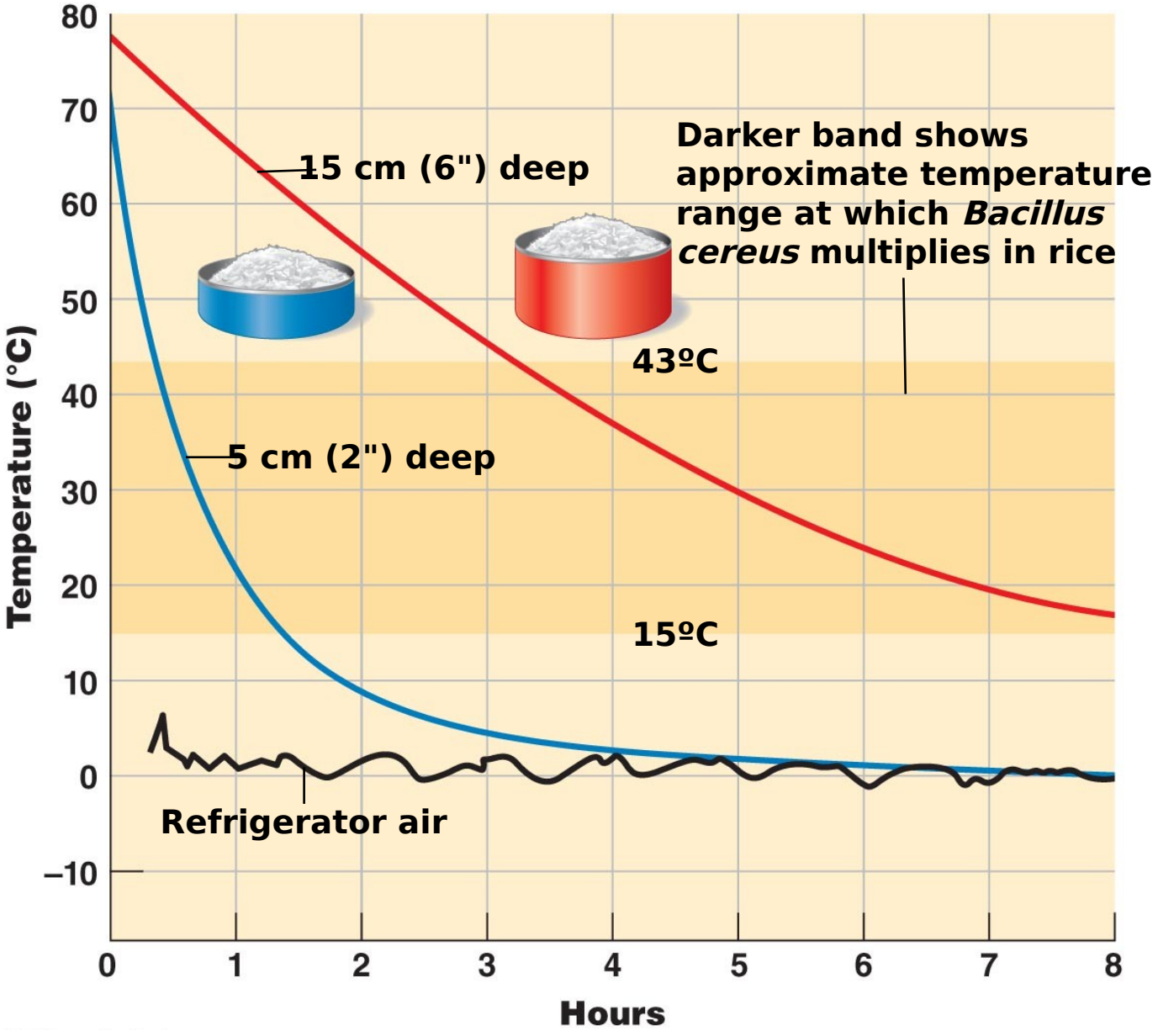


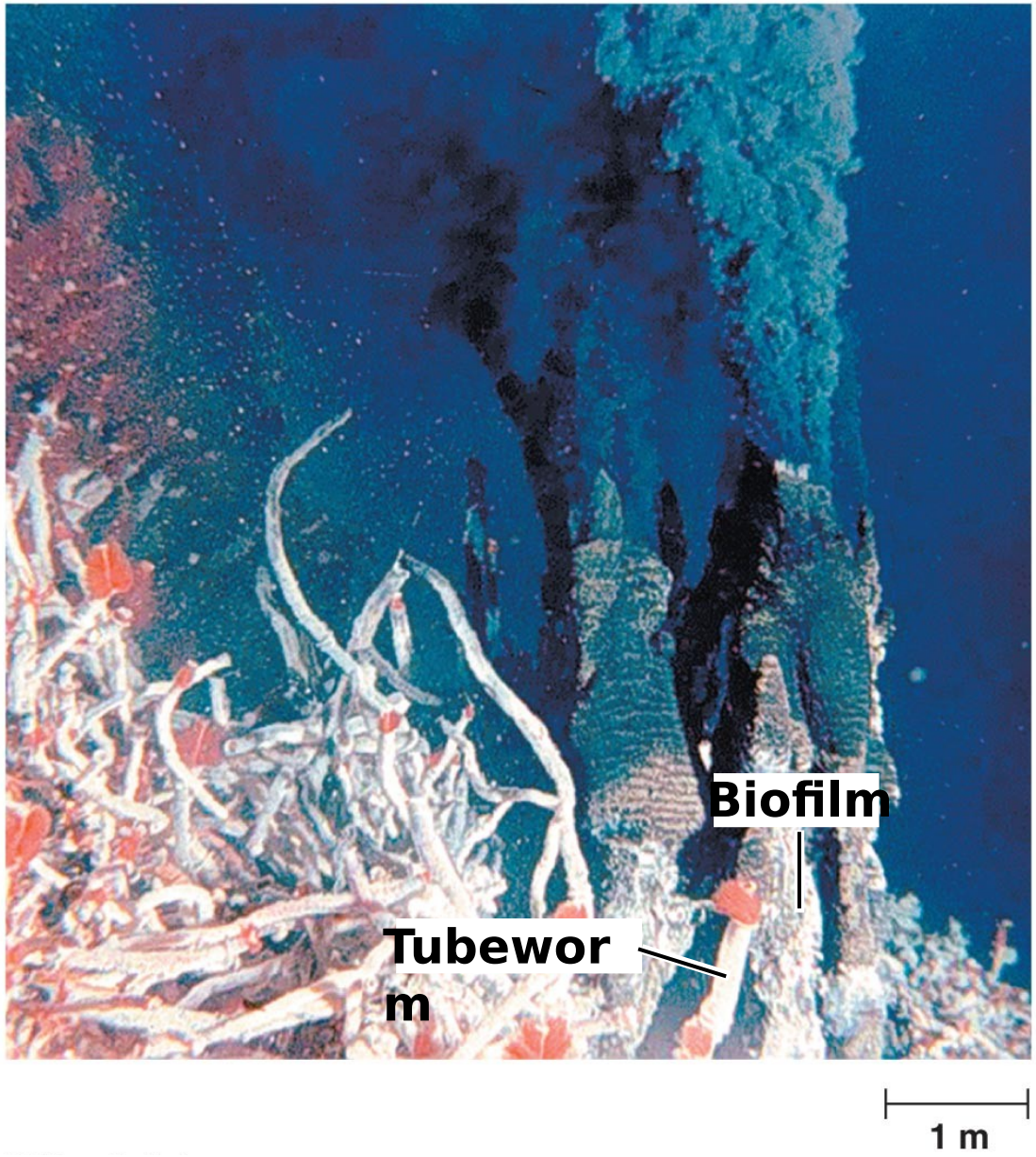
Figure 6.3 The effect of the amount of food on its cooling rate in a refrigerator and its chance of spoilage.



Temperature

- **Thermophiles**
  - Optimum growth temperature of 50 to 60°C
  - Found in hot springs and organic compost
- **Hyperthermophiles**
  - Optimum growth temperature >80°C

Applications of Microbiology 6.1



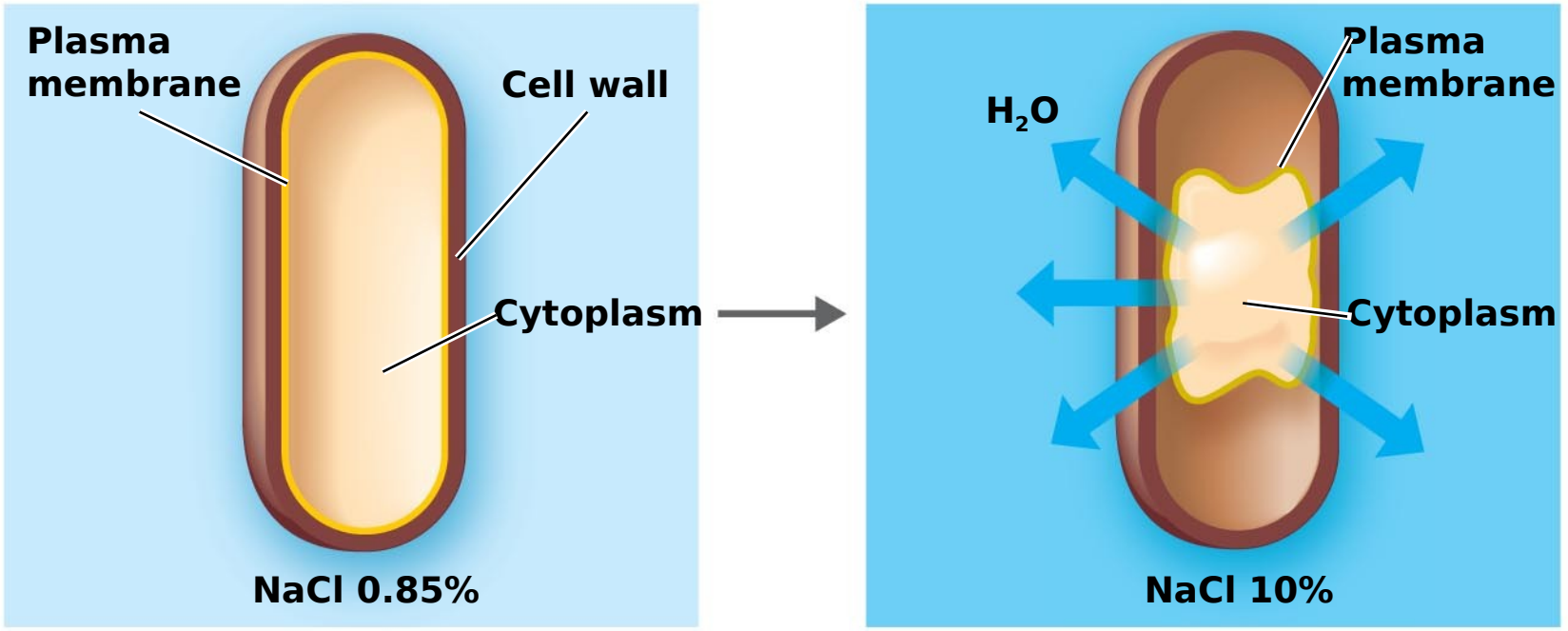
pH

- Most bacteria grow between pH 6.5 and 7.5
- Molds and yeasts grow between pH 5 and 6
- **Acidophiles** grow in acidic environments

Osmotic Pressure

- Hypertonic environments (higher in solutes than inside the cell) cause **plasmolysis** due to high osmotic pressure
- **Extreme** or **obligate halophiles** require high osmotic pressure (high salt)
- **Facultative halophiles** tolerate high osmotic pressure

Figure 6.4 Plasmolysis.



(a) Cell in isotonic solution. **Under these conditions, the solute concentration in the cell is equivalent to a solute concentration of 0.85% sodium chloride (NaCl).**

(b) Plasmolyzed cell in hypertonic solution. **If the concentration of solutes such as NaCl is higher in the surrounding medium than in the cell (the environment is hypertonic), water tends to leave the cell. Growth of the cell is inhibited.**

Check Your Understanding

- Why are hyperthermophiles that grow at temperatures above 100°C seemingly limited to oceanic depths?
  6-1
- Other than controlling acidity, what is an advantage of using phosphate salts as buffers in growth media?
  6-2
- Why might primitive civilizations have used food preservation techniques that rely on osmotic pressure?
  6-3

The Requirements for Growth

Learning Objectives

6-4 Name a use for each of the four elements (carbon, nitrogen, sulfur, and phosphorus) needed in large amounts for microbial growth.

6-5 Explain how microbes are classified on the basis of oxygen requirements.

6-6 Identify ways in which aerobes avoid damage by toxic forms of oxygen.

Chemical Requirements

- Carbon
  - Structural backbone of organic molecules
  - Chemoheterotrophs use organic molecules as energy
  - Autotrophs use CO_2

Chemical Requirements

- Nitrogen
  - Component of proteins, DNA, and ATP
  - Most bacteria decompose protein material for the nitrogen source
  - Some bacteria use NH_4^+ or NO_3^- from organic material
  - A few bacteria use N_2 in **nitrogen fixation**

Chemical Requirements

- Sulfur
  - Used in amino acids, thiamine, and biotin
  - Most bacteria decompose protein for the sulfur source
  - Some bacteria use SO_4^{2-} or H_2S
- Phosphorus
  - Used in DNA, RNA, and ATP
  - Found in membranes
  - PO_4^{3-} is a source of phosphorus

Trace Elements

- Inorganic elements required in small amounts
- Usually as enzyme cofactors
- Include iron, copper, molybdenum, and zinc

Oxygen

- **Obligate aerobes**—require oxygen
- **Facultative anaerobes**—grow via fermentation or anaerobic respiration when oxygen is not available
- **Obligate anaerobes**—unable to use oxygen and are harmed by it
- **Aerotolerant anaerobes**—tolerate but cannot use oxygen
- **Microaerophiles**—require oxygen concentration lower than air

Table 6.1 The Effect of Oxygen on the Growth of Various Types of Bacteria

TABLE **6.1** The Effect of Oxygen on the Growth of Various Types of Bacteria

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Microaerophiles
Effect of Oxygen on Growth	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; growth ceases in presence of oxygen.	Only anaerobic growth; but growth continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
Bacterial Growth in Tube of Solid Growth Medium					
Explanation of Growth Patterns	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.
Explanation of Oxygen's Effects	Presence of enzymes catalase and superoxide dismutase (SOD) allows toxic forms of oxygen to be neutralized; can use oxygen.	Presence of enzymes catalase and SOD allows toxic forms of oxygen to be neutralized; can use oxygen.	Lacks enzymes to neutralize harmful forms of oxygen; cannot tolerate oxygen.	Presence of one enzyme, SOD, allows harmful forms of oxygen to be partially neutralized; tolerates oxygen.	Produce lethal amounts of toxic forms of oxygen if exposed to normal atmospheric oxygen.

Oxygen

- **Singlet oxygen:** ($^1O_2^-$) boosted to a higher-energy state and is reactive
- **Superoxide radicals:** $\cdot O_2^-$

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

- **Peroxide anion:** O_2^{2-}

$$2\,H_2O_2 \longrightarrow 2\,H_2O + O_2$$

$$H_2O_2 + 2\,H^+ \longrightarrow 2\,H_2O$$

- **Hydroxyl radical** (OH•)

Organic Growth Factors

- Organic compounds obtained from the environment
- Vitamins, amino acids, purines, and pyrimidines

Check Your Understanding

- If bacterial cells were given a sulfur source containing radioactive sulfur (^{35}S) in their culture media, in what molecules would the ^{35}S be found in the cells?
  6-4
- How would one determine whether a microbe is a strict anaerobe?
  6-5
- Oxygen is so pervasive in the environment that it would be very difficult for a microbe to always avoid physical contact with it. What, therefore, is the most obvious way for a microbe to avoid damage?
  6-6

Biofilms

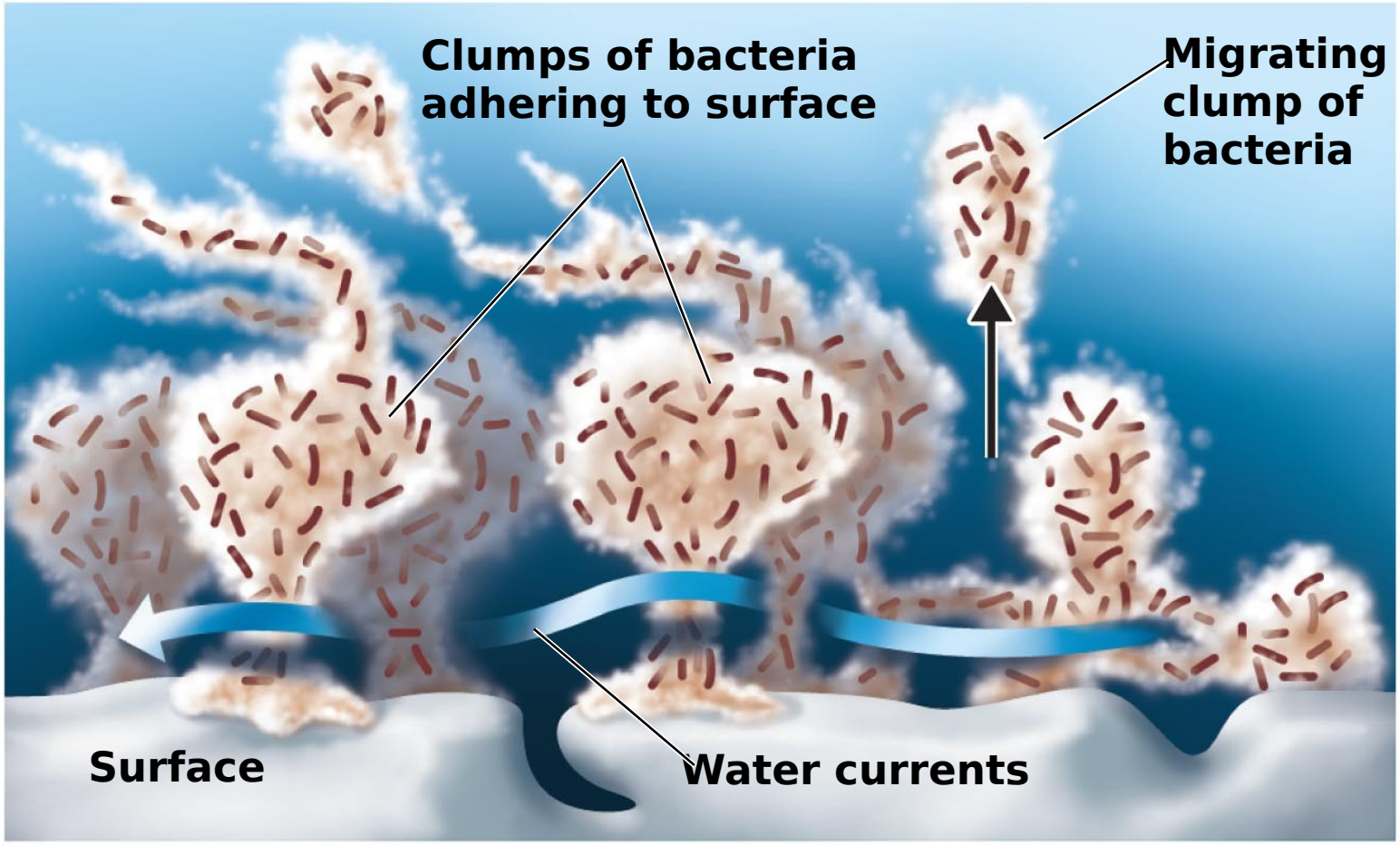
Learning Objective

6-7 Describe the formation of biofilms and their potential for causing infection.

Biofilms

- Microbial communities
- Form slime or hydrogels that adhere to surfaces
  - Bacteria communicate cell-to-cell via quorum sensing
- Share nutrients
- Shelter bacteria from harmful environmental factors

Figure 6.5 Biofilms.

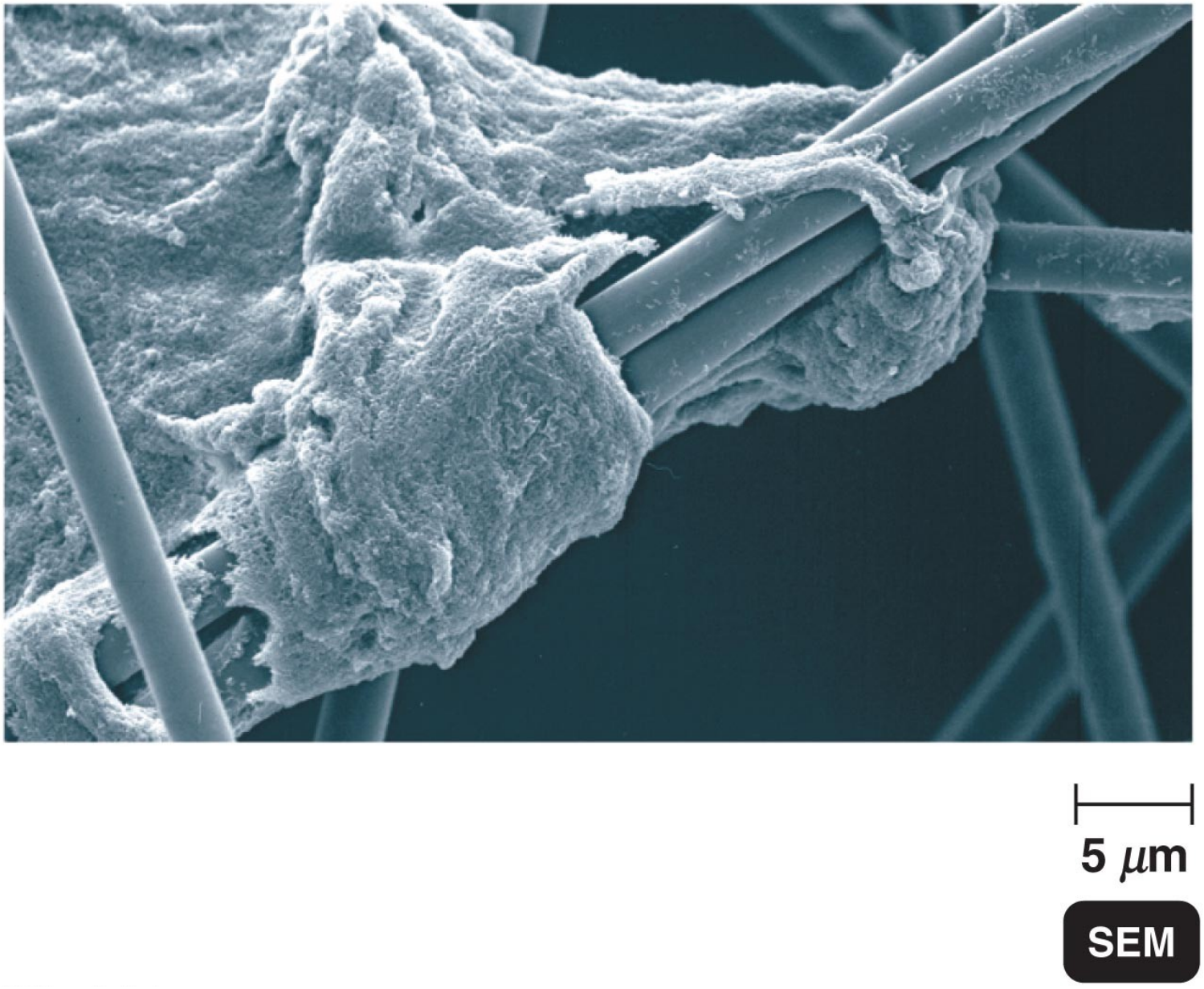


Water currents move, as shown by the blue arrow, among pillars of slime formed by the growth of bacteria attached to solid surfaces. This allows efficient access to nutrients and removal of bacterial waste products. Individual slime-forming bacteria or bacteria in clumps of slime detach and move to new locations.

Biofilms

- Found in digestive system and sewage treatment systems; can clog pipes
- 1000x resistant to microbicides
- Involved in 70% of infections
  - Catheters, heart valves, contact lenses, dental caries

Applications of Microbiology 3.1b



Check Your Understanding

- ✓ Identify a way in which pathogens find it advantageous to form biofilms.
  6-7

Culture Media

Learning Objectives

6-8 Distinguish chemically defined and complex media.

6-9 Justify the use of each of the following: anaerobic techniques, living host cells, candle jars, selective and differential media, enrichment medium.

6-10 Differentiate biosafety levels 1, 2, 3, and 4.

Culture Media

- **Culture medium:** nutrients prepared for microbial growth
- **Sterile:** no living microbes
- **Inoculum:** introduction of microbes into a medium
- **Culture:** microbes growing in or on a culture medium

Culture Media

- **Agar**
  - Complex polysaccharide
  - Used as a solidifying agent for culture media in Petri plates, slants, and deeps
  - Generally not metabolized by microbes
  - Liquefies at 100°C
  - Solidifies at ~40°C

Culture Media

- **Chemically defined media:** exact chemical composition is known
  - Fastidious organisms are those that require many growth factors provided in chemically defined media
- **Complex media:** extracts and digests of yeasts, meat, or plants; chemical composition varies batch to batch
  - **Nutrient broth**
  - **Nutrient agar**

Table 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Table 6.3 Defined Culture Medium for *Leuconostoc mesenteroides*

TABLE 6.3 Defined Culture Medium for *Leuconostoc mesenteroides*

Carbon and Energy
Glucose, 25 g
Salts
NH_4Cl, 3.0 g
K_2HPO_4*, 0.6 g
KH_2PO_4*, 0.6 g
$MgSO_4$, 0.1 g
Amino Acids, 100–200 µg each
Alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Purines and Pyrimidines, 10 mg of each
Adenine, guanine, uracil, xanthine
Vitamins, 0.01–1 mg each
Biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid
Trace Elements, 2–10 µg each
Fe, Co, Mn, Zn, Cu, Ni, Mo
Buffer, pH 7
Sodium acetate, 25 g
Distilled Water, 1000 ml
*Also serves as buffer.

Table 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Anaerobic Growth Media and Methods

- **Reducing media**
  - Used for the cultivation of anaerobic bacteria
  - Contain chemicals (sodium thioglycolate) that combine O_2 to deplete it
  - Heated to drive off O_2

Figure 6.6 A jar for cultivating anaerobic bacteria on Petri plates.

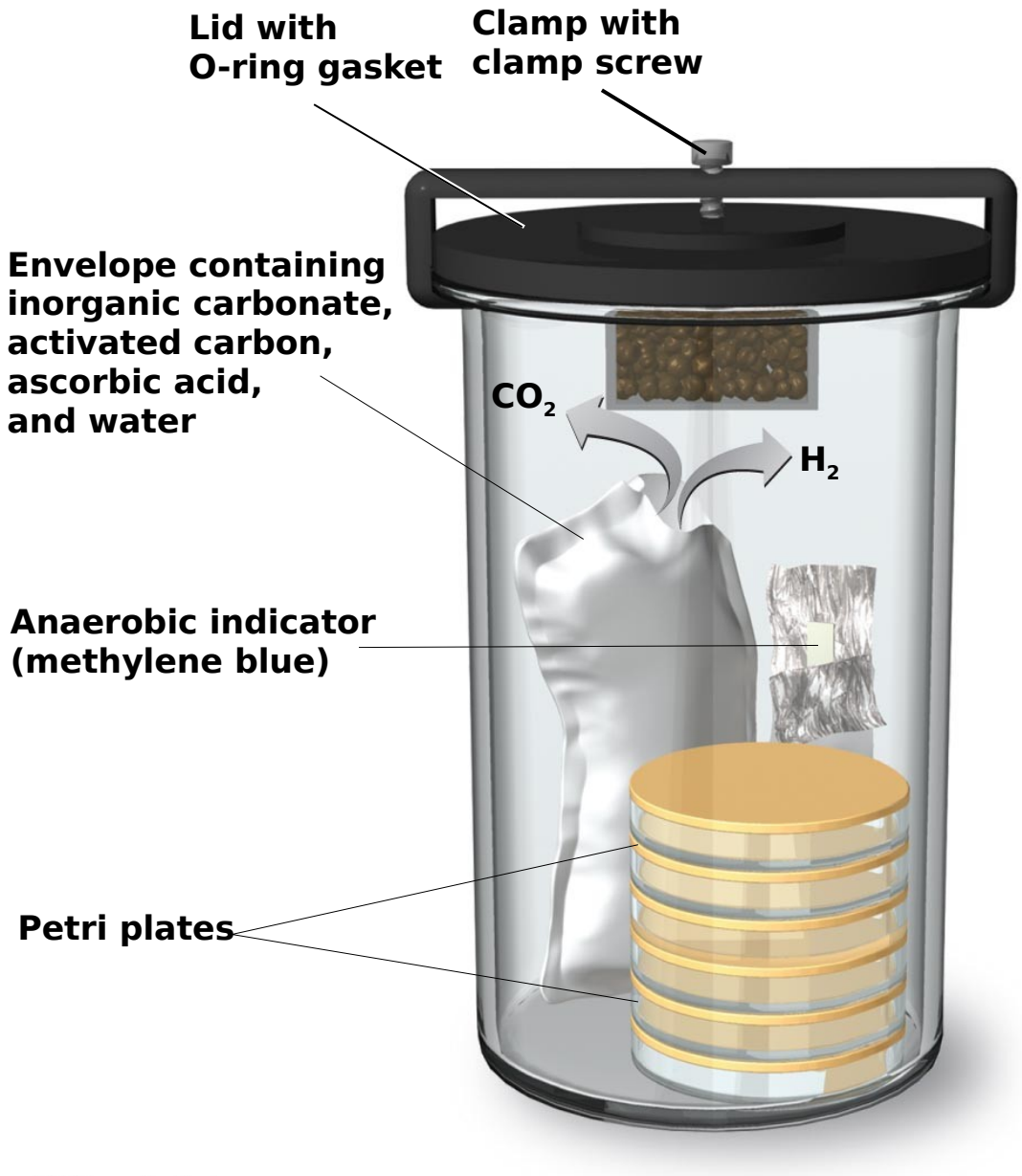
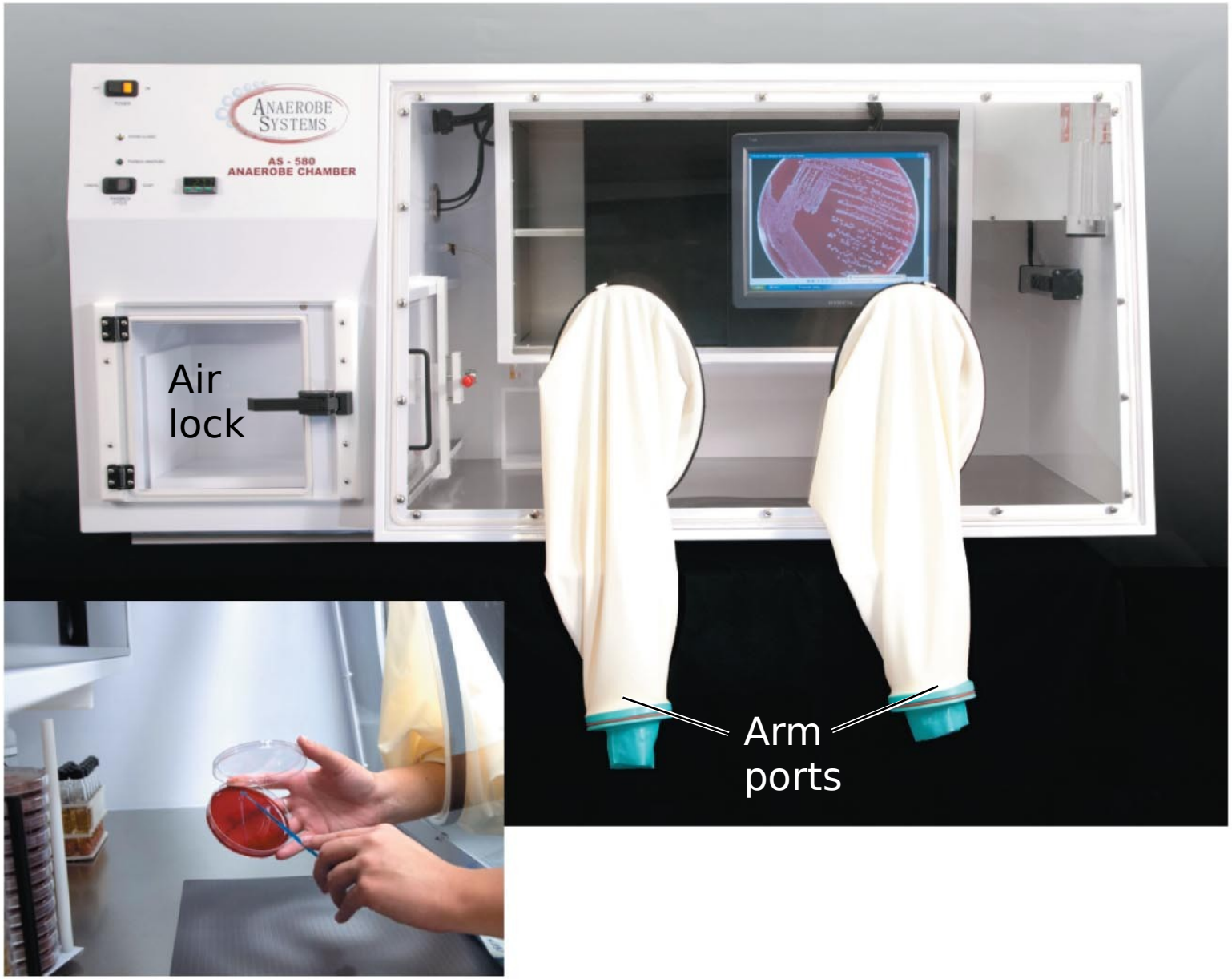


Figure 6.7 An anaerobic chamber.



Special Culture Techniques

- **Capnophiles**
  - Microbes that require high CO_2 conditions
  - CO_2 packet
  - Candle jar

Special Culture Techniques

- Biosafety levels
  - BSL-1: no special precautions; basic teaching labs
  - BSL-2: lab coat, gloves, eye protection
  - BSL-3: biosafety cabinets to prevent airborne transmission
  - BSL-4: sealed, negative pressure; "hot zone"
    - Exhaust air is filtered twice through HEPA filters

Figure 6.8 Technicians in a biosafety level 4 (BSL-4) laboratory.

Selective and Differential Media

- **Selective media**
  - Suppress unwanted microbes and encourage desired microbes
  - Contain inhibitors to suppress growth

Selective and Differential Media

- **Differential media**
  - Allow distinguishing of colonies of different microbes on the same plate
- Some media have both selective and differential characteristics

Figure 6.9 Blood agar, a differential medium containing red blood cells.

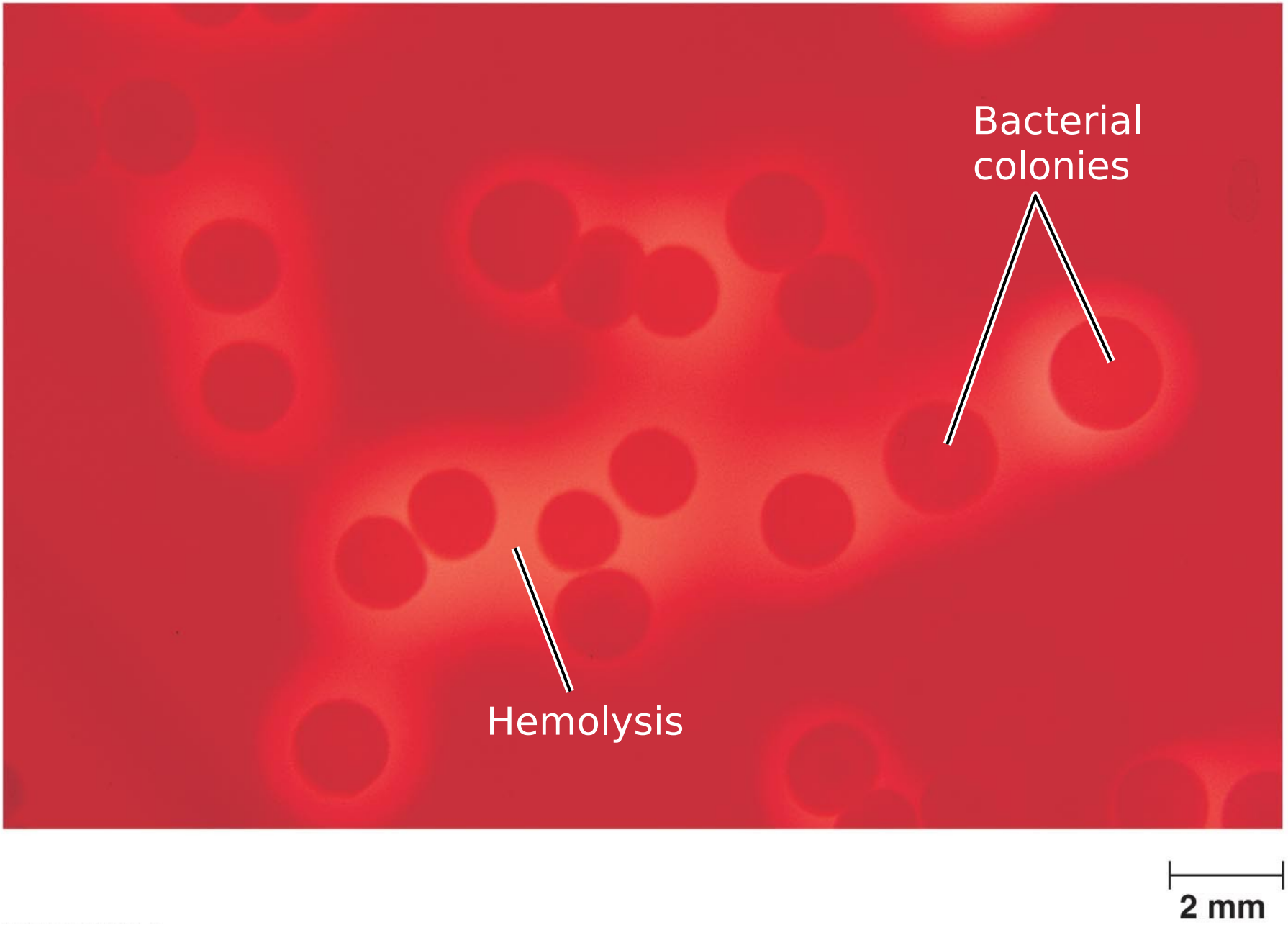
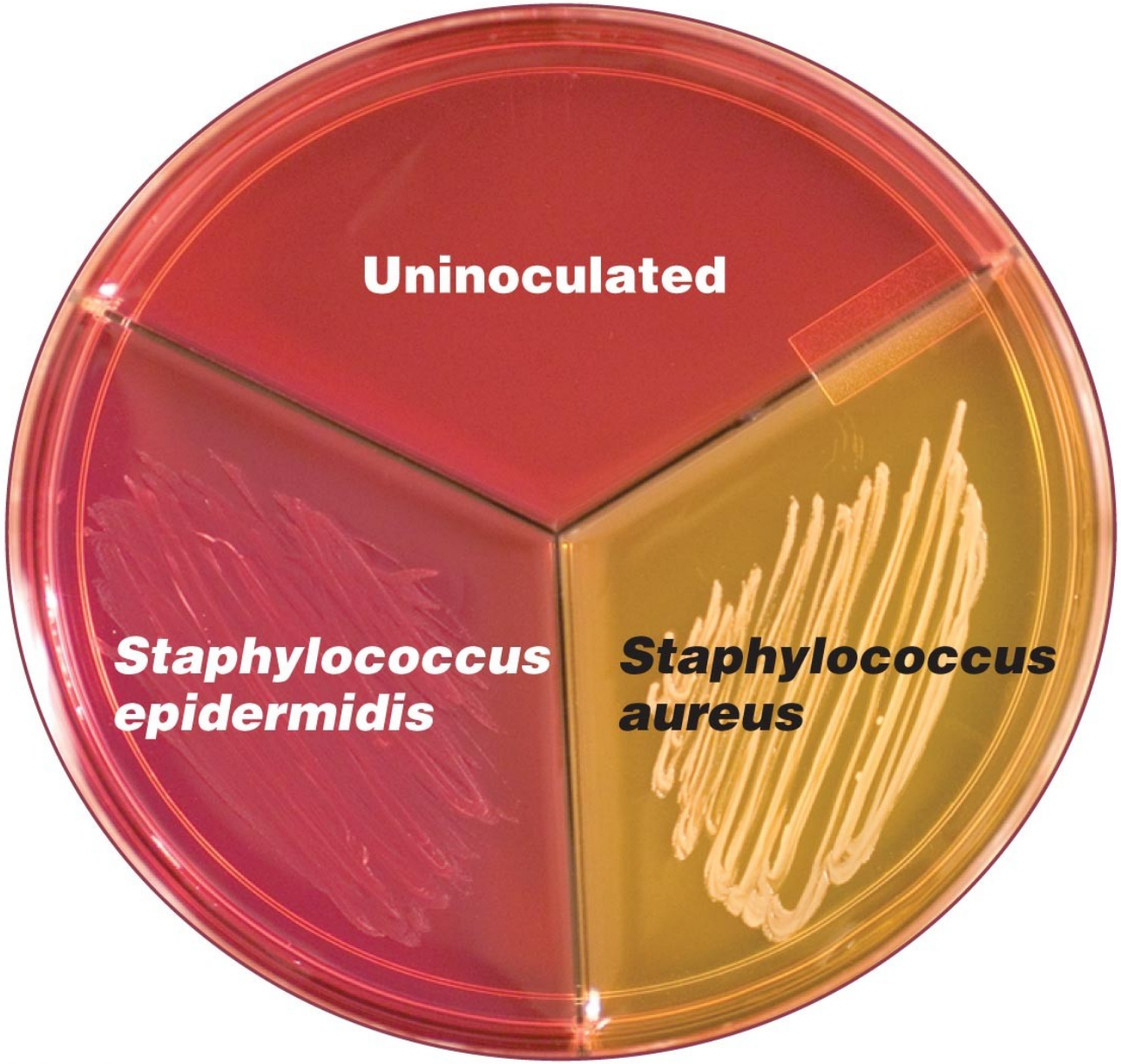


Figure 6.10 Differential medium.



Enrichment Culture

- Encourages the growth of a desired microbe by increasing very small numbers of a desired organism to detectable levels
- Usually a liquid

TABLE 6.5 Culture Media

Type	Purpose
Chemically Defined	Growth of chemoautotrophs and photoautotrophs; microbiological assays
Complex	Growth of most chemoheterotrophic organisms
Reducing	Growth of obligate anaerobes
Selective	Suppression of unwanted microbes; encouraging desired microbes
Differential	Differentiation of colonies of desired microbes from others
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels

Check Your Understanding

- ✓ Could humans exist on chemically defined media,
  at least under laboratory conditions?
  6-8
- ✓ Could Louis Pasteur, in the 1800s, have grown rabies viruses in cell culture instead of in living animals?
  6-9
- ✓ What BSL is your laboratory?
  6-10

Obtaining Pure Cultures

Learning Objectives

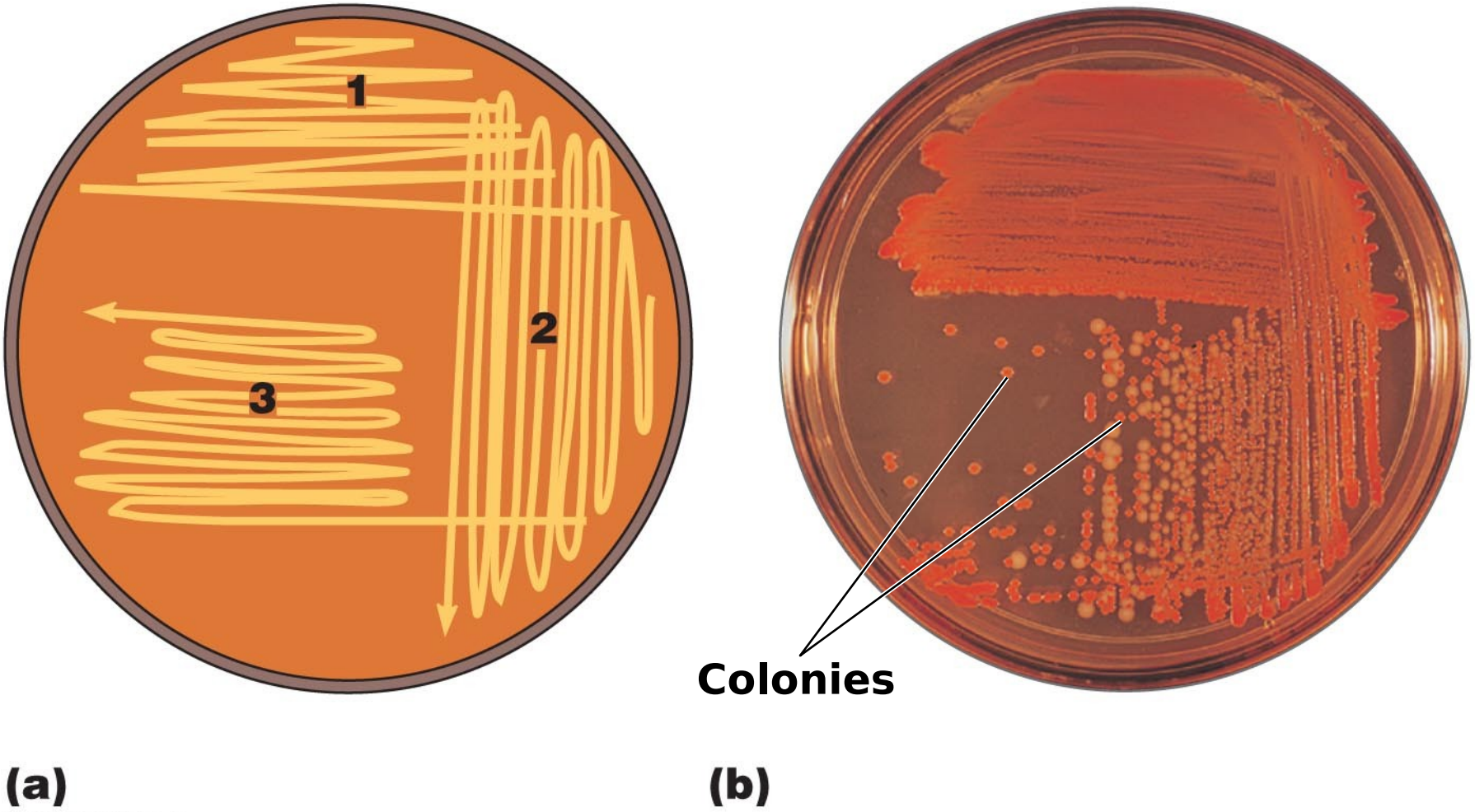
6-11 Define *colony.*

6-12 Describe how pure cultures can be isolated by using the streak plate method.

Obtaining Pure Cultures

- A pure culture contains only one species or strain
- A **colony** is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a **colony-forming unit** (CFU)
- The **streak plate method** is used to isolate pure cultures

Figure 6.11 The streak plate method for isolating pure bacterial cultures.



Check Your Understanding

- ✓ Can you think of any reason why a colony does not grow to an infinite size, or at least fill the confines of the Petri plate?
  6-11
- ✓ Could a pure culture of bacteria be obtained by the streak plate method if there were only one desired microbe in a bacterial suspension of billions?
  6-12

Preserving Bacterial Cultures

Learning Objective

6-13 Explain how microorganisms are preserved by deep-freezing and lyophilization (freeze-drying).

Preserving Bacterial Cultures

- **Deep-freezing:** –50° to –95°C
- **Lyophilization (freeze-drying):** frozen (–54° to –72°C) and dehydrated in a vacuum

Check Your Understanding

- If the Space Station in Earth orbit suddenly ruptured, the humans on board would die instantly from cold and the vacuum of space. Would all the bacteria in the capsule also be killed?
  6-13

The Growth of Bacterial Cultures

Learning Objectives

6-14 Define *bacterial growth,* including *binary fission.*

6-15 Compare the phases of microbial growth, and describe their relation to generation time.

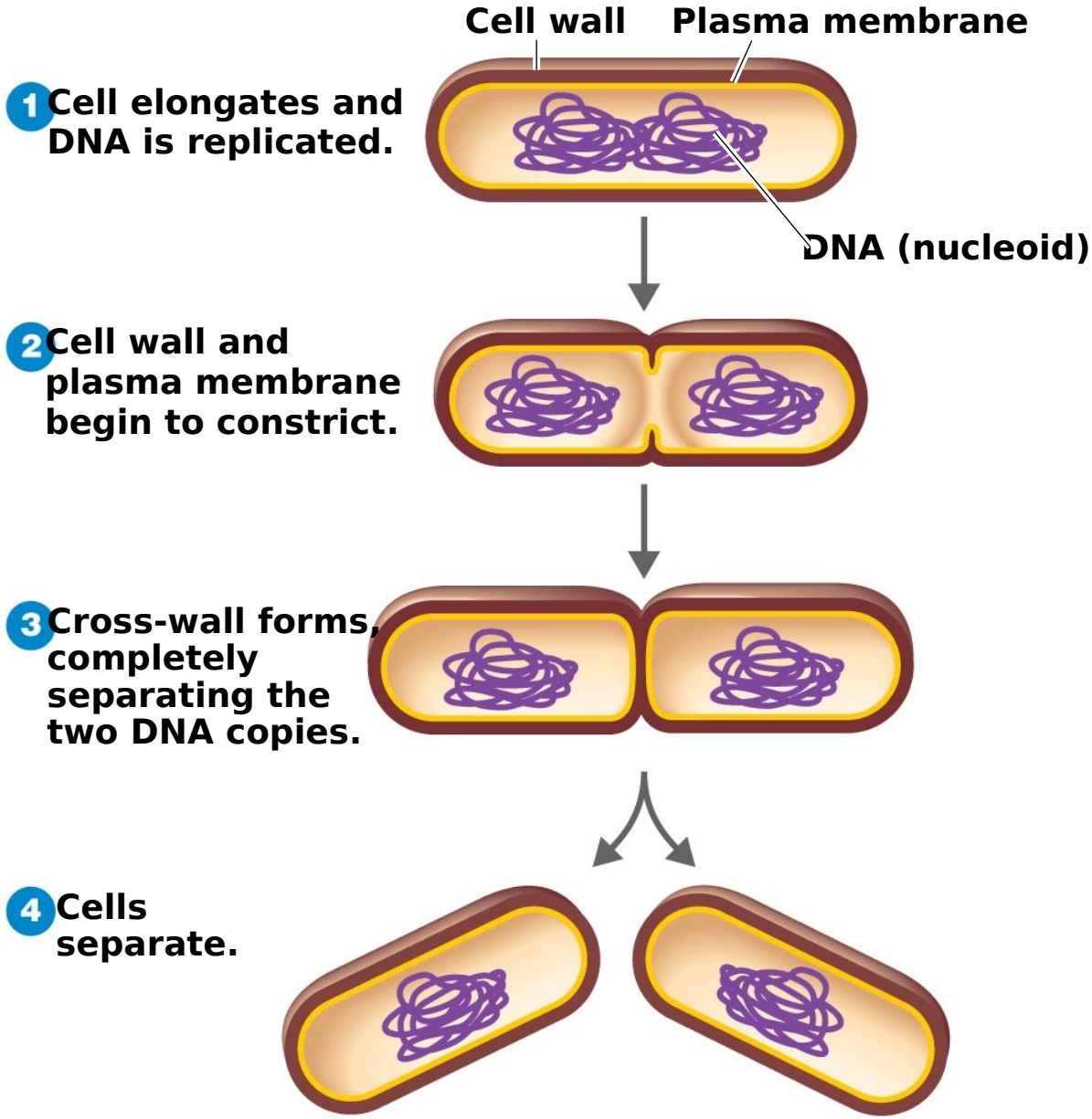
Bacterial Division

- Increase in number of cells, not cell size
- **Binary fission**
- **Budding**
- Conidiospores (actinomycetes)
- Fragmentation of filaments

Bacterial Growth: Overview

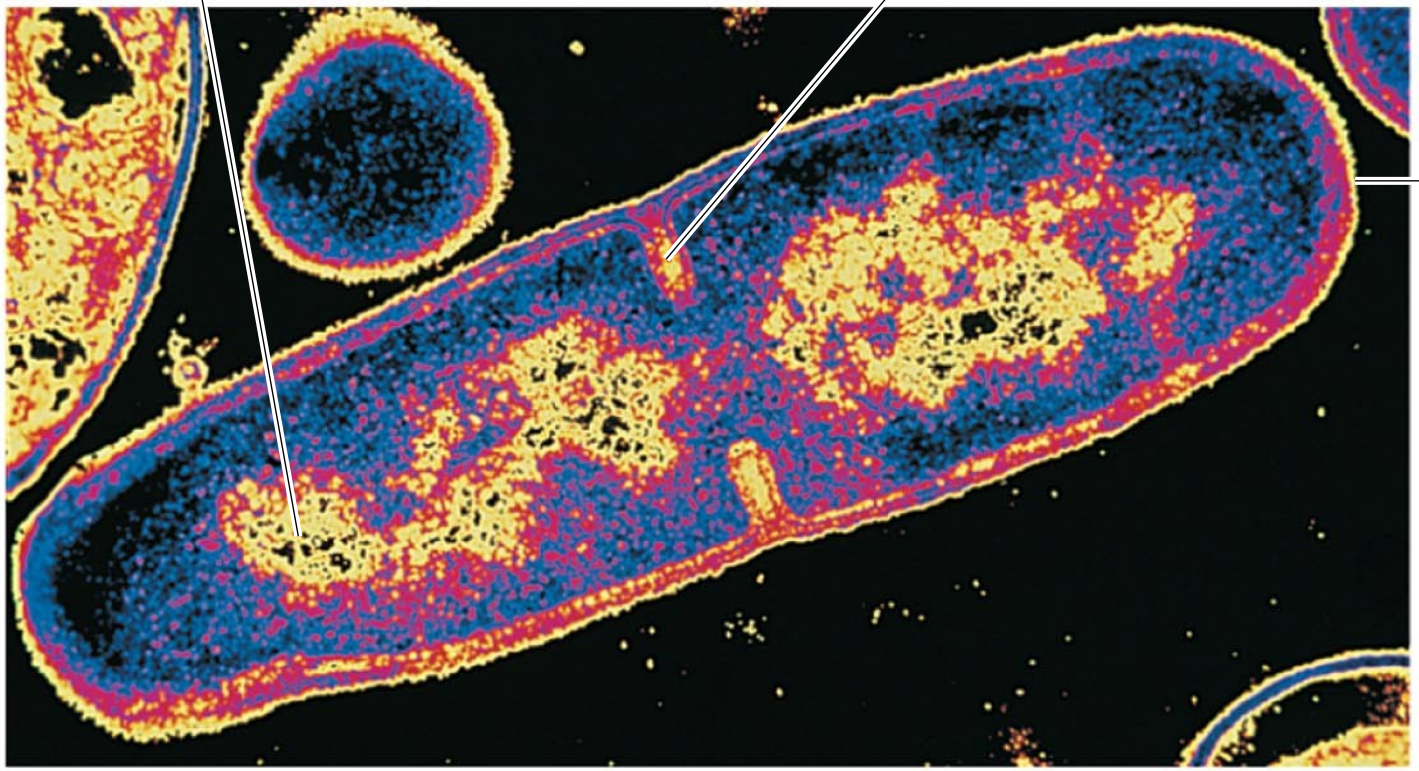
PLAY **Animation: Bacterial Growth: Overview**

Figure 6.12a Binary fission in bacteria.



(a) A diagram of the sequence of cell division

Figure 6.12b Binary fission in bacteria.

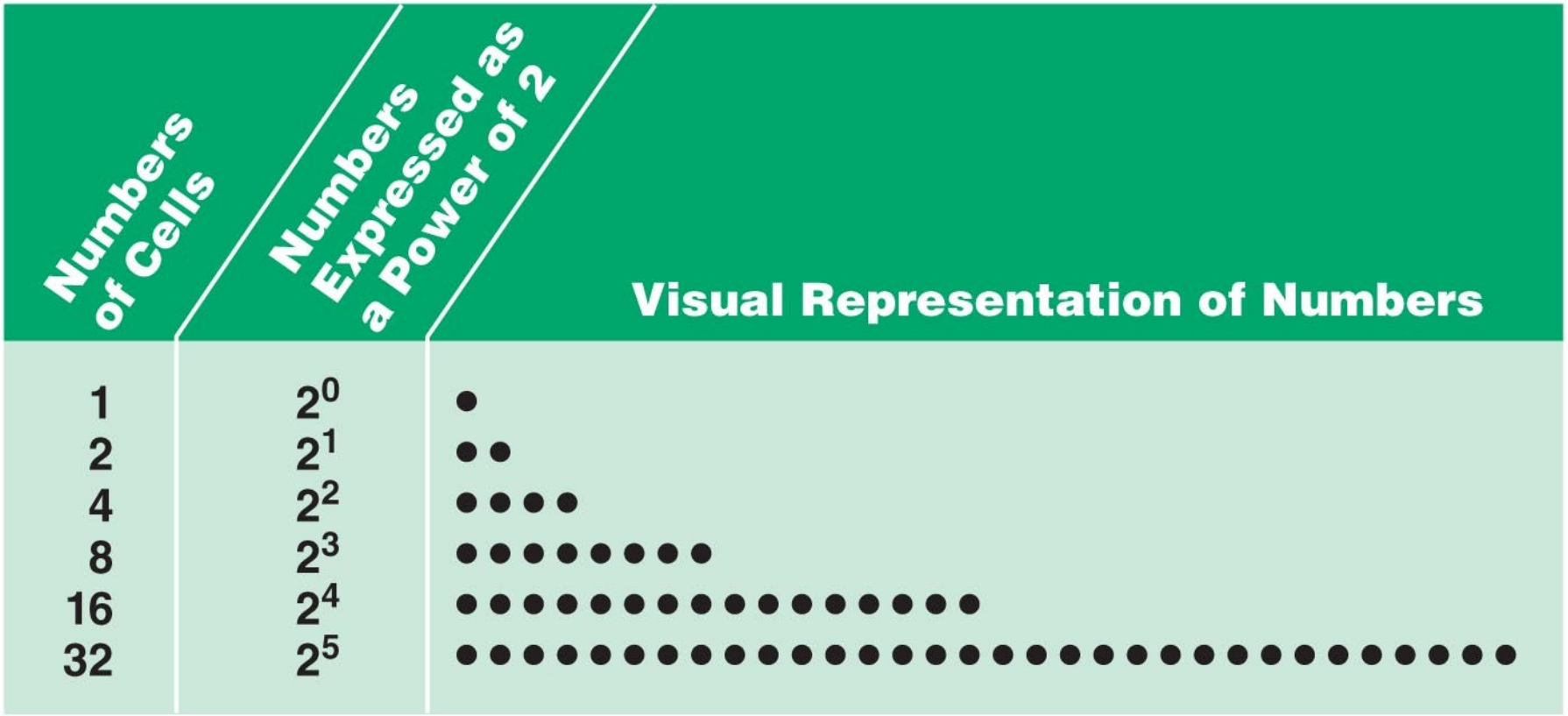


(B) A thin section of a cell of *Bacillus licheniformis* starting to divide

Generation Time

- Time required for a cell to divide
  - 20 minutes to 24 hours
- Binary fission doubles the number of cells each generation
- Total number of cells = $2^{\text{number of generations}}$
- Growth curves are represented logarithmically

Figure 6.13a Cell division.



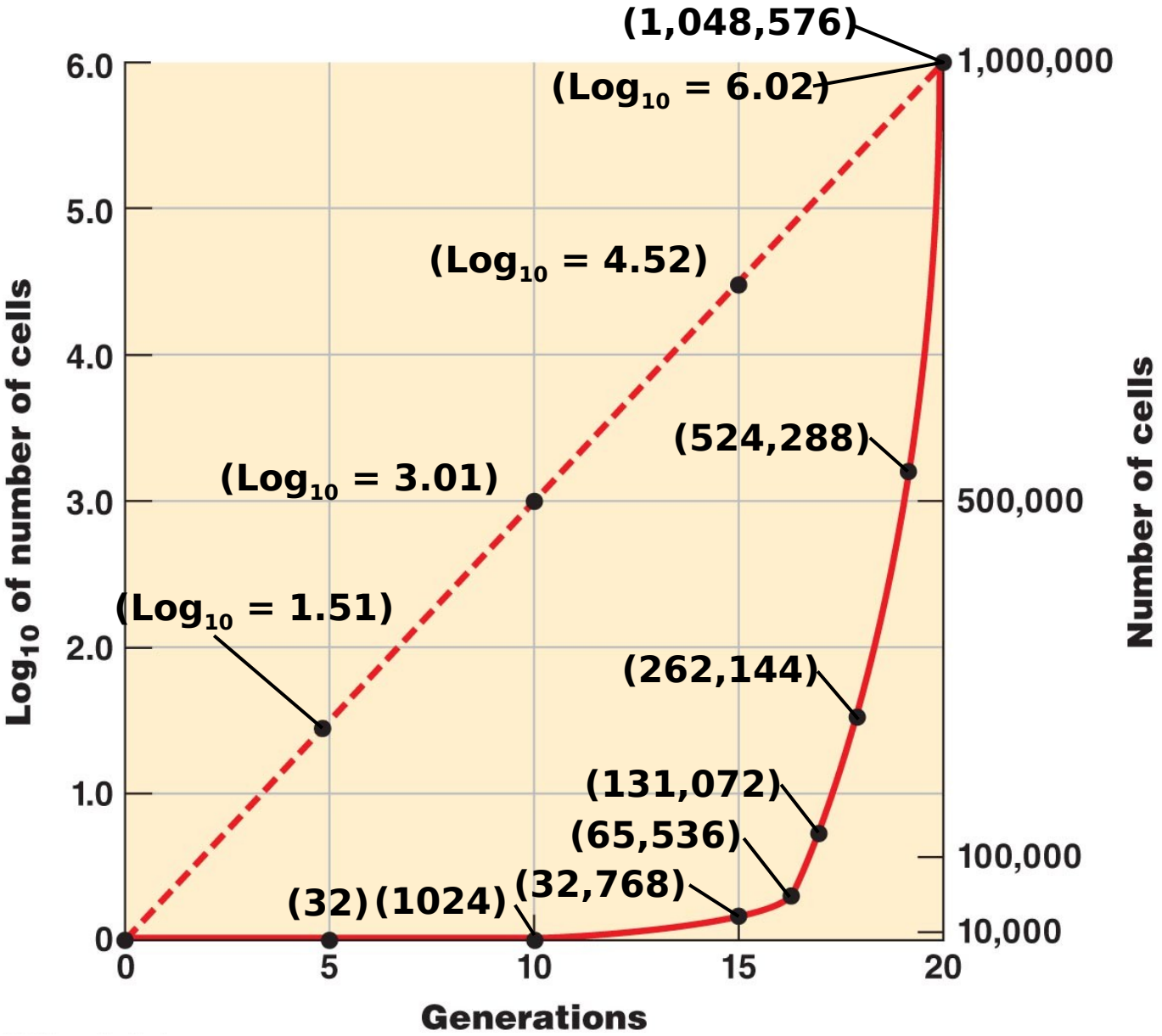
(a) Visual representation of increase in bacterial number over five generations. The number of bacteria doubles in each generation. The superscript indicates the generation; that is, $2^5 = 5$ generations.

Figure 6.13b Cell division.

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

(b) Conversion of the number of cells in a population into the logarithmic expression of this number. To arrive at the numbers in the center column, use the y^x key on your calculator. Enter 2 on the calculator; press y^x; enter 5; then press the = sign. The calculator will show the number 32. Thus, the fifth-generation population of bacteria will total 32 cells. To arrive at the numbers in the right-hand column, use the log key on your calculator. Enter the number 32; then press the log key. The calculator will show, rounded off, that the $\log_{10}$ of 32 is 1.51.

Figure 6.14 A growth curve for an exponentially increasing population, plotted logarithmically (dashed line) and arithmetically (solid line).



Binary Fission

Animation: Binary Fission

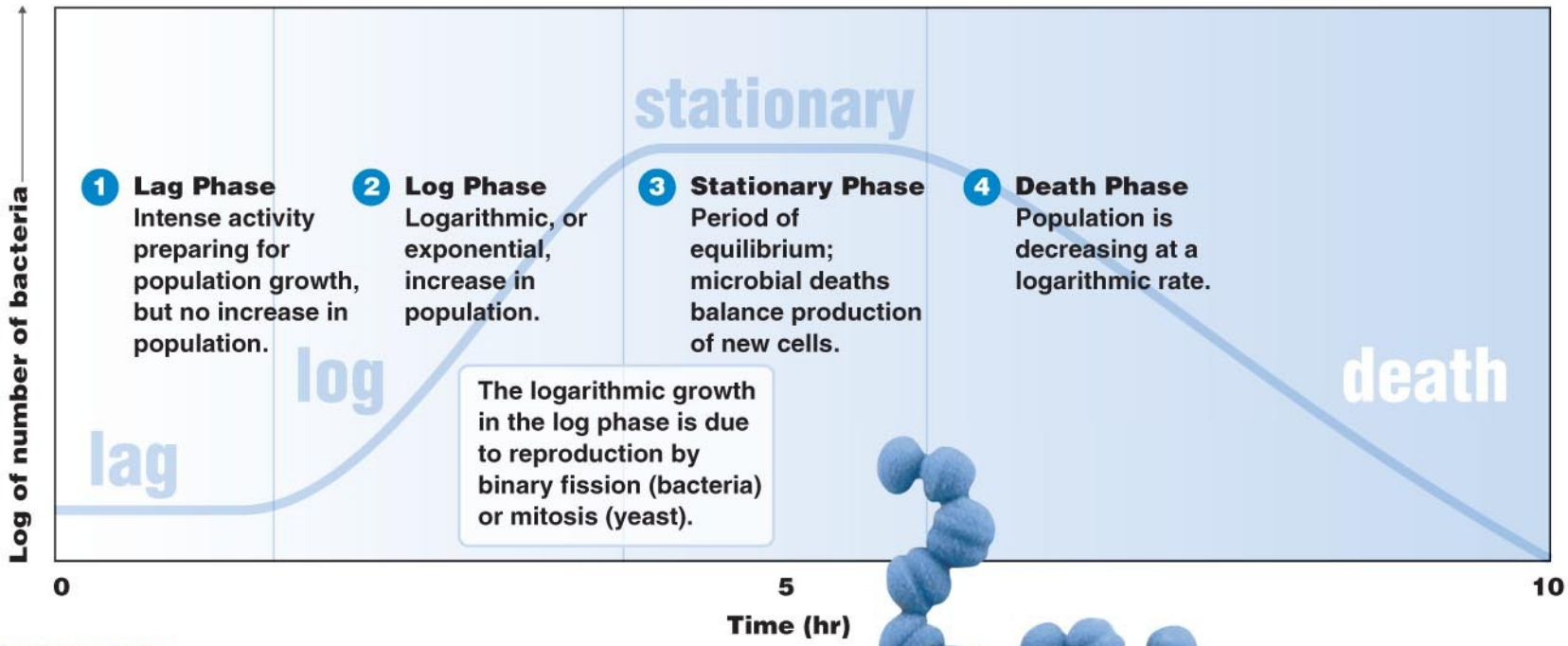
Check Your Understanding

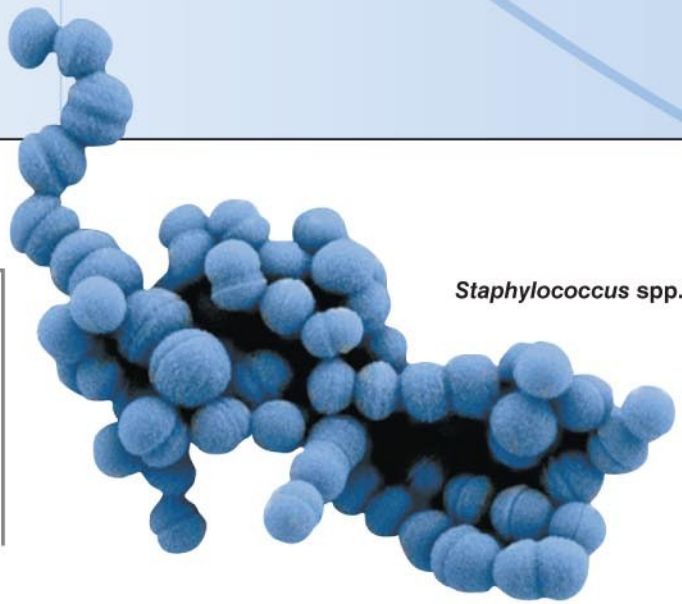
- ✓ Can a complex organism, such as a beetle, divide by binary fission?
  6-14

Phases of Growth

- **Lag phase**
- **Log phase**
- **Stationary phase**
- **Death phase**

Figure 6.15 Understanding the Bacterial Growth Curve.





KEY CONCEPTS

Bacterial populations follow a sequential series of growth phases: the lag, log, stationary, and death phases.

Knowledge of the bacterial growth curve is critical to understanding population dynamics and population control in the course of infectious diseases, in food preservation and spoilage, as well as in industrial microbiology processes, such as ethanol production.

Bacterial Growth Curve

PLAY **nimation: Bacterial Growth Curve**

Check Your Understanding

- If two mice started a family within a fixed enclosure, with a fixed food supply, would the population curve be the same as a bacterial growth curve?
  6-15

The Growth of Bacterial Cultures

Learning Objectives

6-16 Explain four direct methods of measuring cell growth.

6-17 Differentiate direct and indirect methods of measuring cell growth.

6-18 Explain three indirect methods of measuring cell growth.

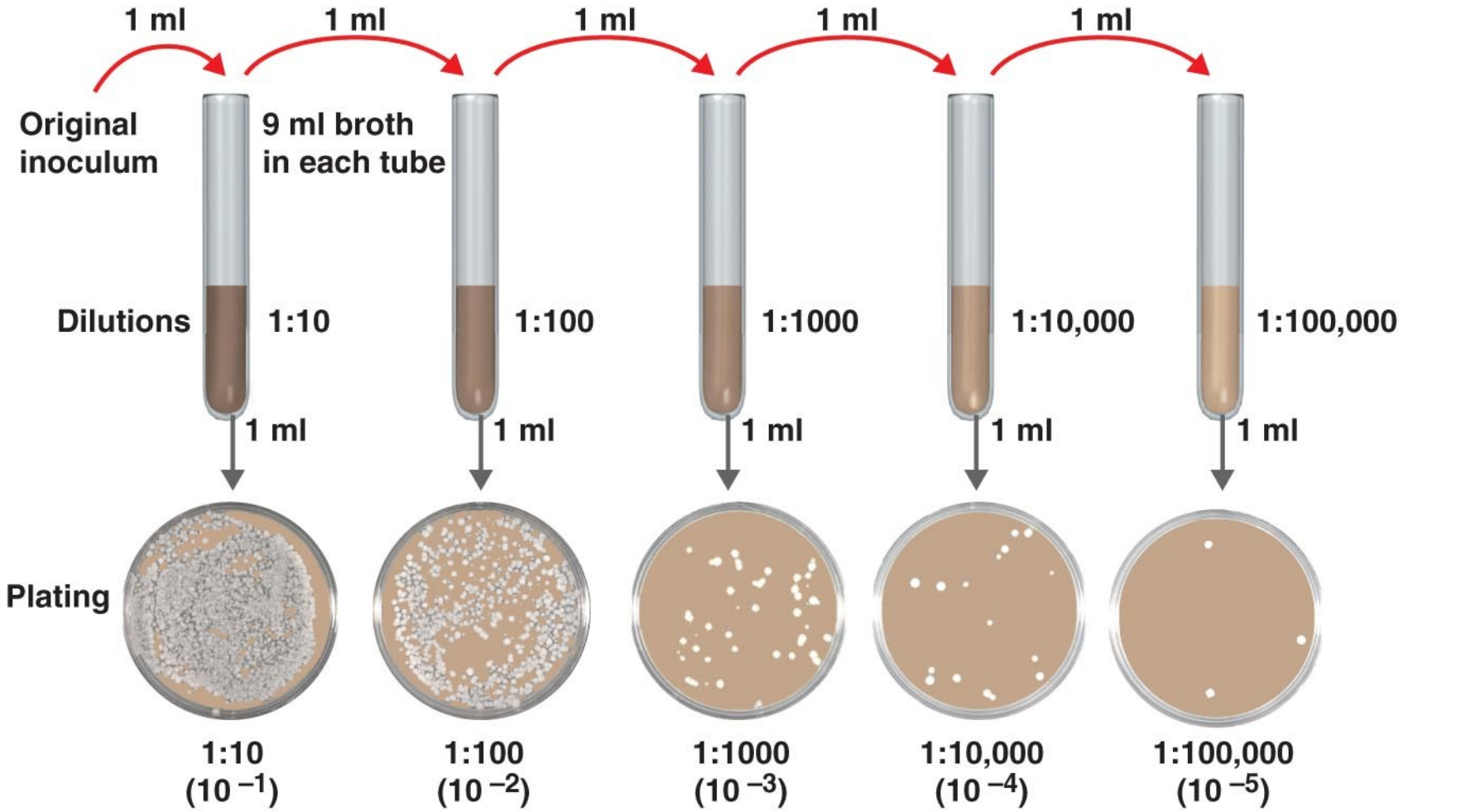
Direct Measurement of Microbial Growth

- Direct measurements–count microbial cells
  - **Plate count**
  - **Filtration**
  - **Most probable number (MPN) method**
  - **Direct microscopic count**

Plate Counts

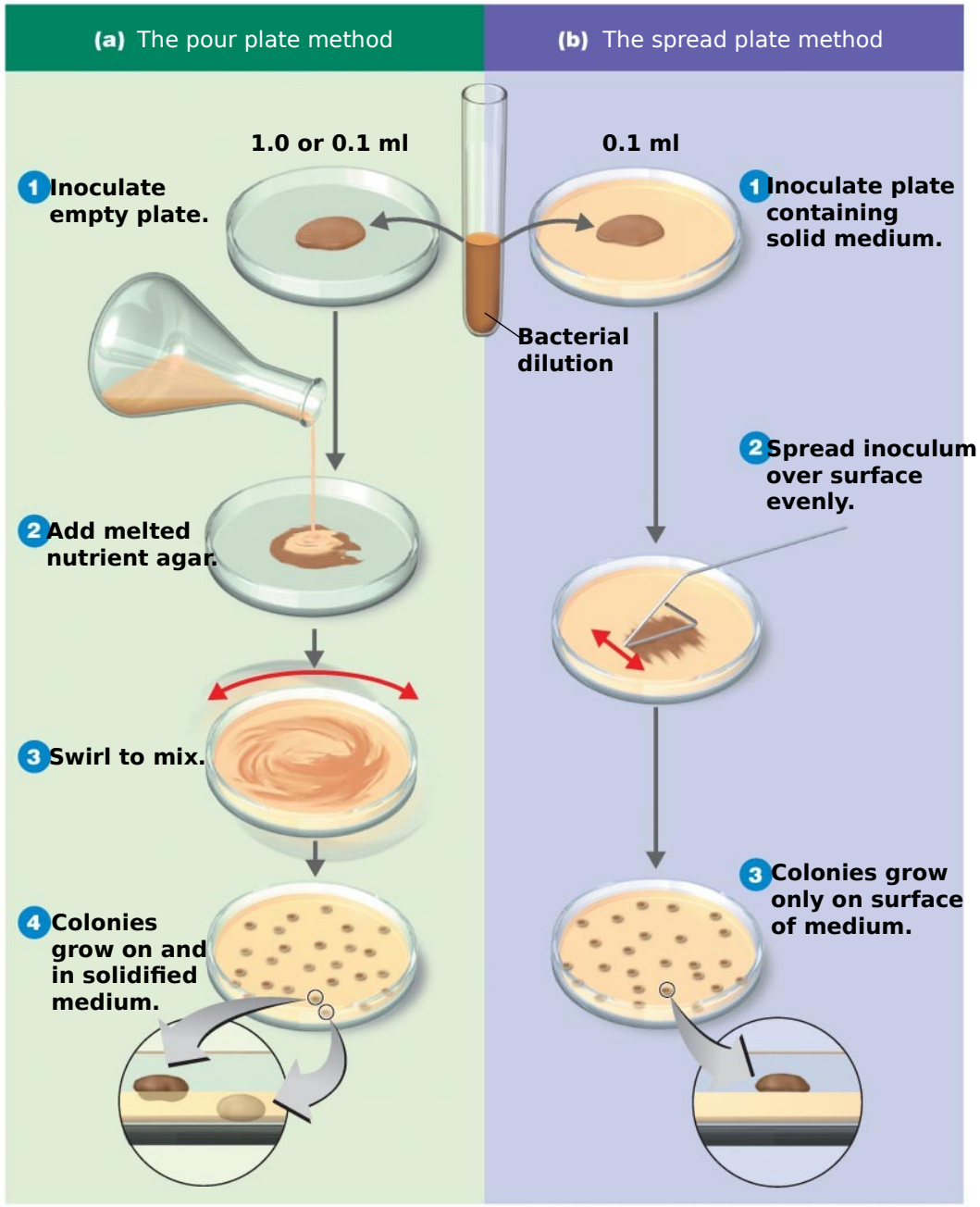
- Count colonies on plates that have 30 to 300 colonies (CFUs)
- To ensure the right number of colonies, the original inoculum must be diluted via **serial dilution**
- Counts are performed on bacteria mixed into a dish with agar **(pour plate method)** or spread on the surface of a plate **(spread plate method)**

Figure 6.16 Serial dilutions and plate counts.



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 54 colonies are on a plate of 1:1000 dilution, then the count is 54 × 1000 = 54,000 bacteria/ml in sample.)

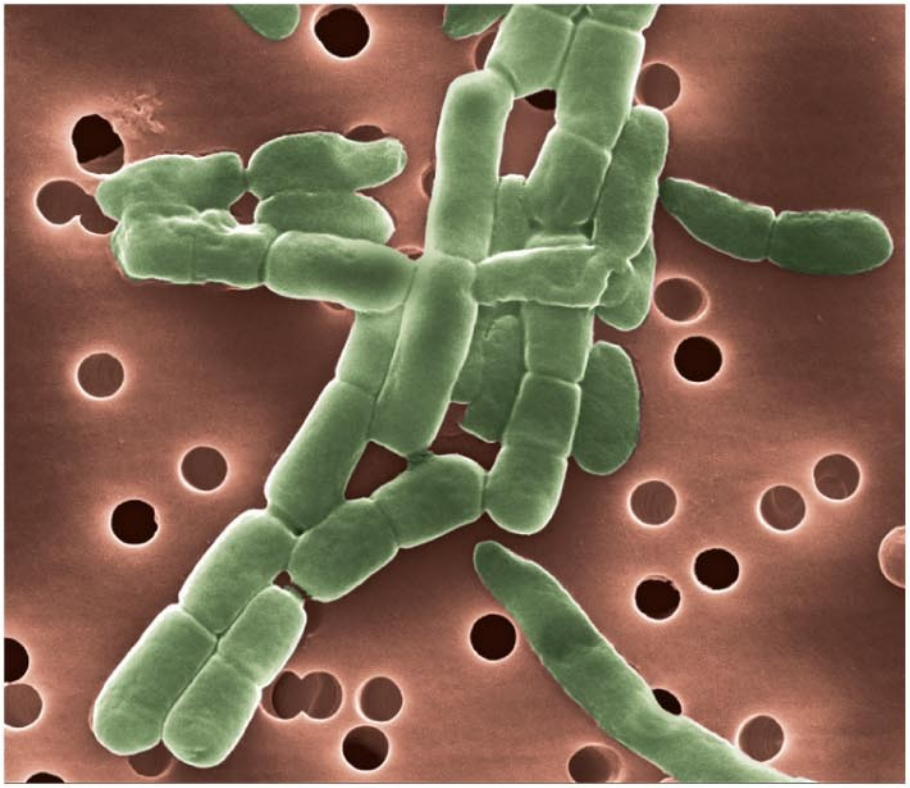
Figure 6.17 Methods of preparing plates for plate counts.



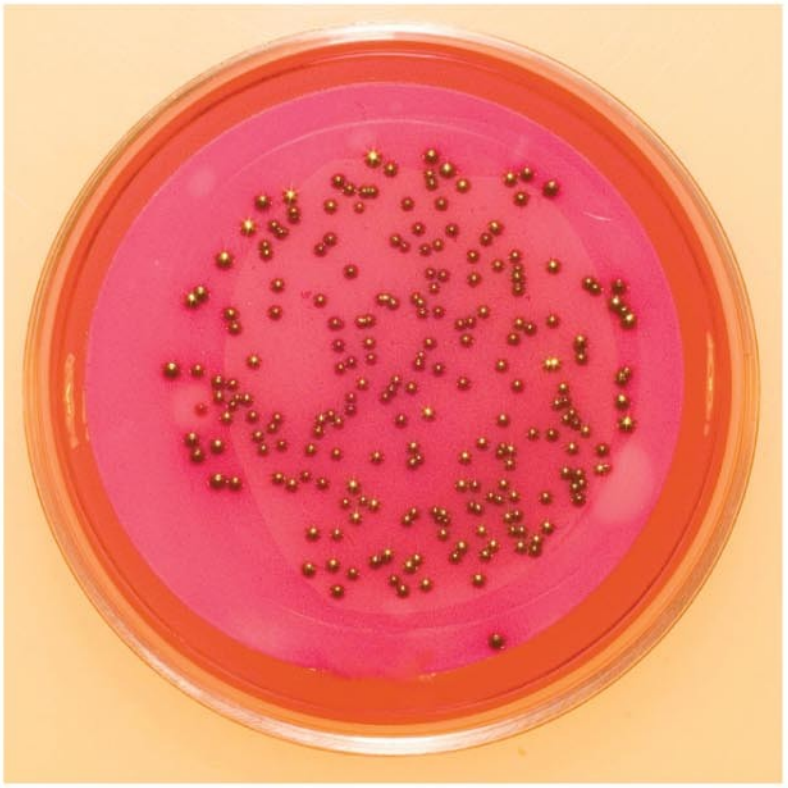
Filtration

- Solution passed through a filter that collects bacteria
- Filter is transferred to a Petri dish and grows as colonies on the surface

Figure 6.18 Counting bacteria by filtration.



(a) The bacterial populations in bodies of water can be determined by passing a sample through a membrane filter. Here, the bacteria in a 100-ml water sample have been sieved out onto the surface of a membrane filter. These bacteria form visible colonies when placed on the surface of a suitable medium.

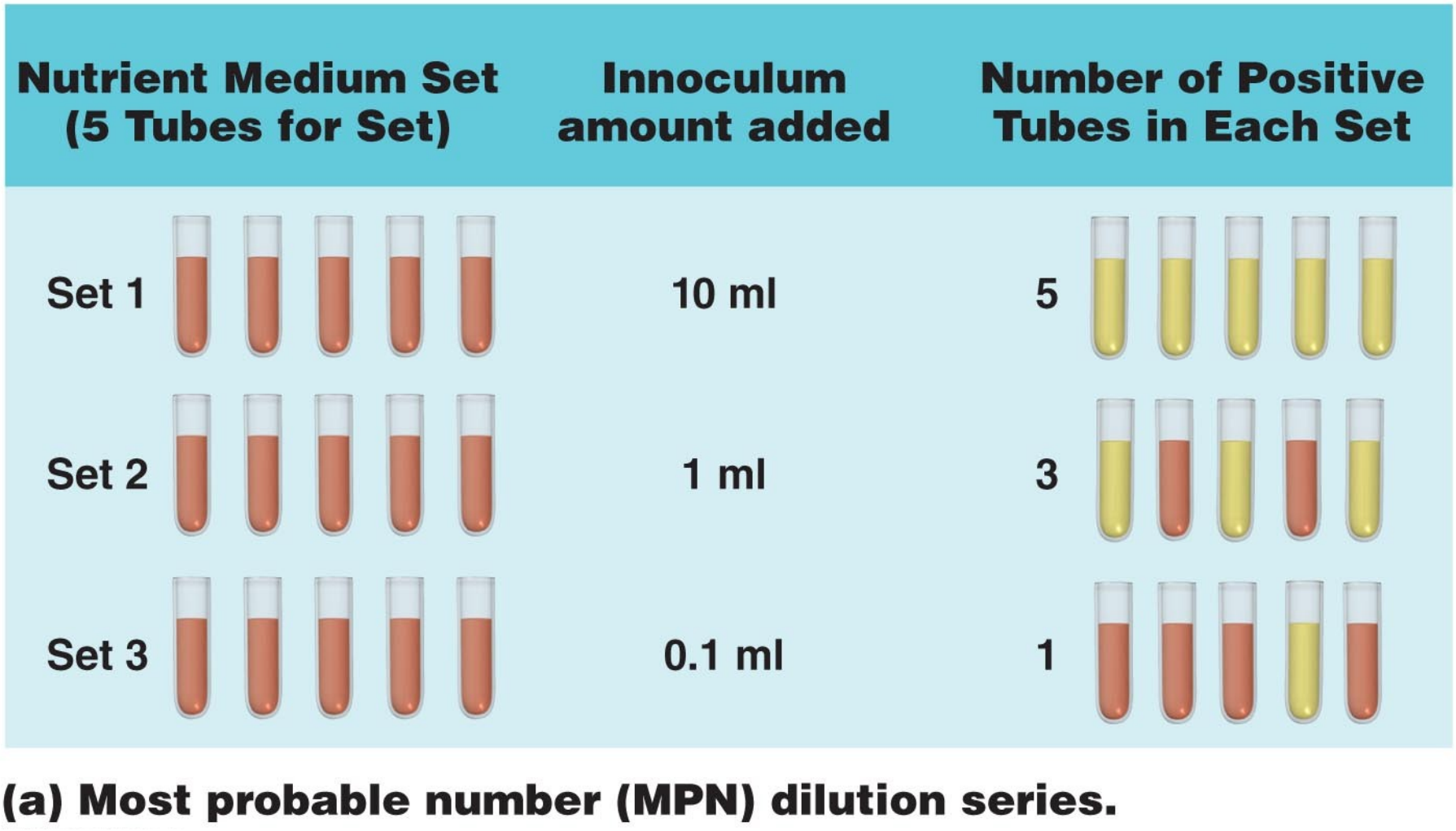


(b) A membrane filter with bacteria on its surface, as described in (a), has been placed on Endo agar. This medium is selective for gram-negative bacteria; lactose fermenters, such as the coliforms, form distinctive colonies. There are 214 colonies visible, so we would record 214 bacteria per 100 ml in the water sample.

The Most Probable Number (MPN) Method

- Multiple tube test
- Count positive tubes
- Compare with a statistical table

Figure 6.19a The most probable number (MPN) method.



(a) Most probable number (MPN) dilution series.

Figure 6.19b The most probable number (MPN) method.

Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	6.8	50
4-2-1	26	9.8	70
4-3-0	27	9.9	70
4-3-1	33	10	70
4-4-0	34	14	100
5-0-0	23		70
5-0-1	31	10	70
5-0-2	43	14	100
5-1-0	33	10	100
5-1-1	46	14	120
5-1-2	63	22	150
5-2-0	49	15	150
5-2-1	70	22	170
5-2-2	94	34	230
5-3-0	79	22	220
5-3-1	110	34	250
5-3-2	140	52	400

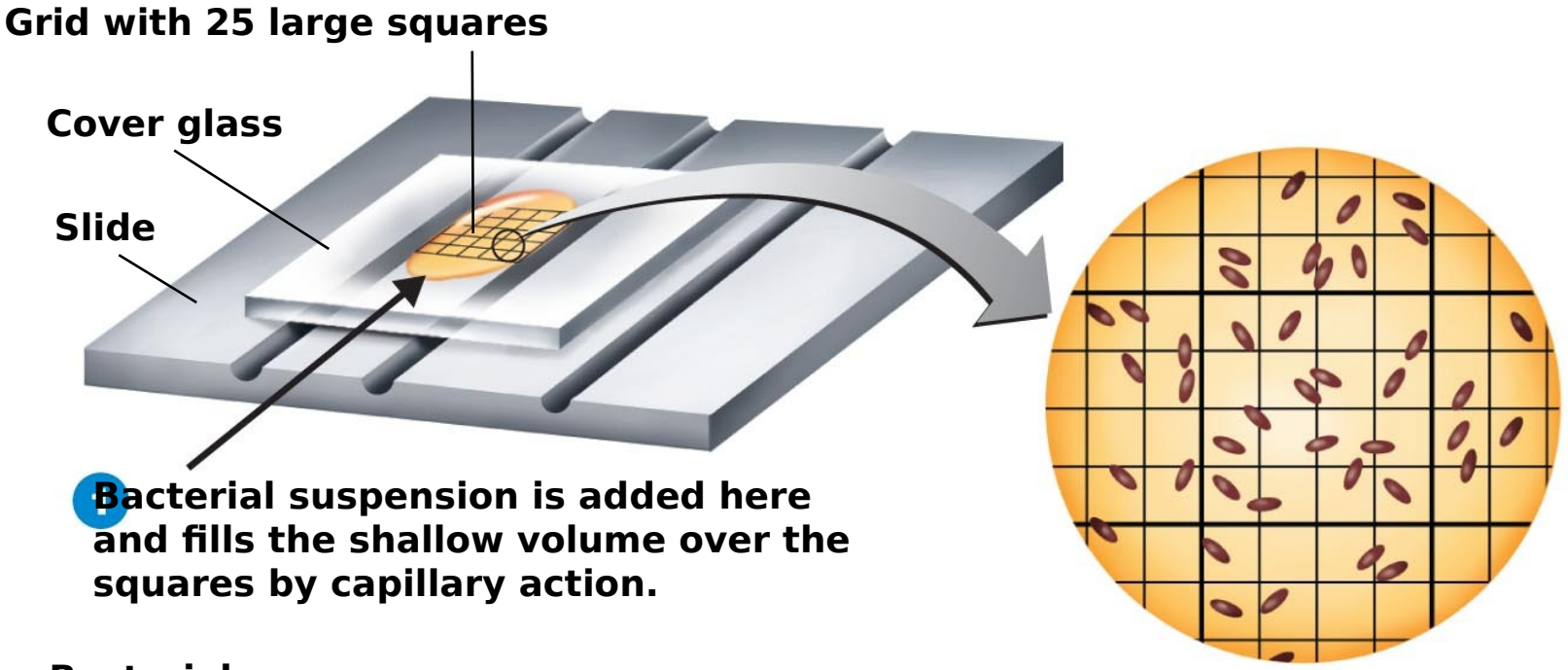
(b) MPN table. MPN tables enable us to calculate for a sample the microbial numbers that are statistically likely to lead to such a result. The number of positive (yellow) tubes is recorded for each set: in the shaded example, 5, 3, and 1. If we look up this combination in an MPN table, we find that the MPN index per 100 ml is 110. Statistically, this means that 95% of the water samples that give this result contain 34–250 bacteria, with 110 being the most probable number.

Direct Microscopic Count

- Volume of a bacterial suspension placed on a slide
- Average number of bacteria per viewing field is calculated
- Uses a special Petroff-Hausser cell counter

$$\text{Number of bacteria/ml} = \frac{\text{Number of cells counted}}{\text{Volume of area counted}}$$

Figure 6.20 Direct microscopic count of bacteria with a Petroff-Hausser cell counter.



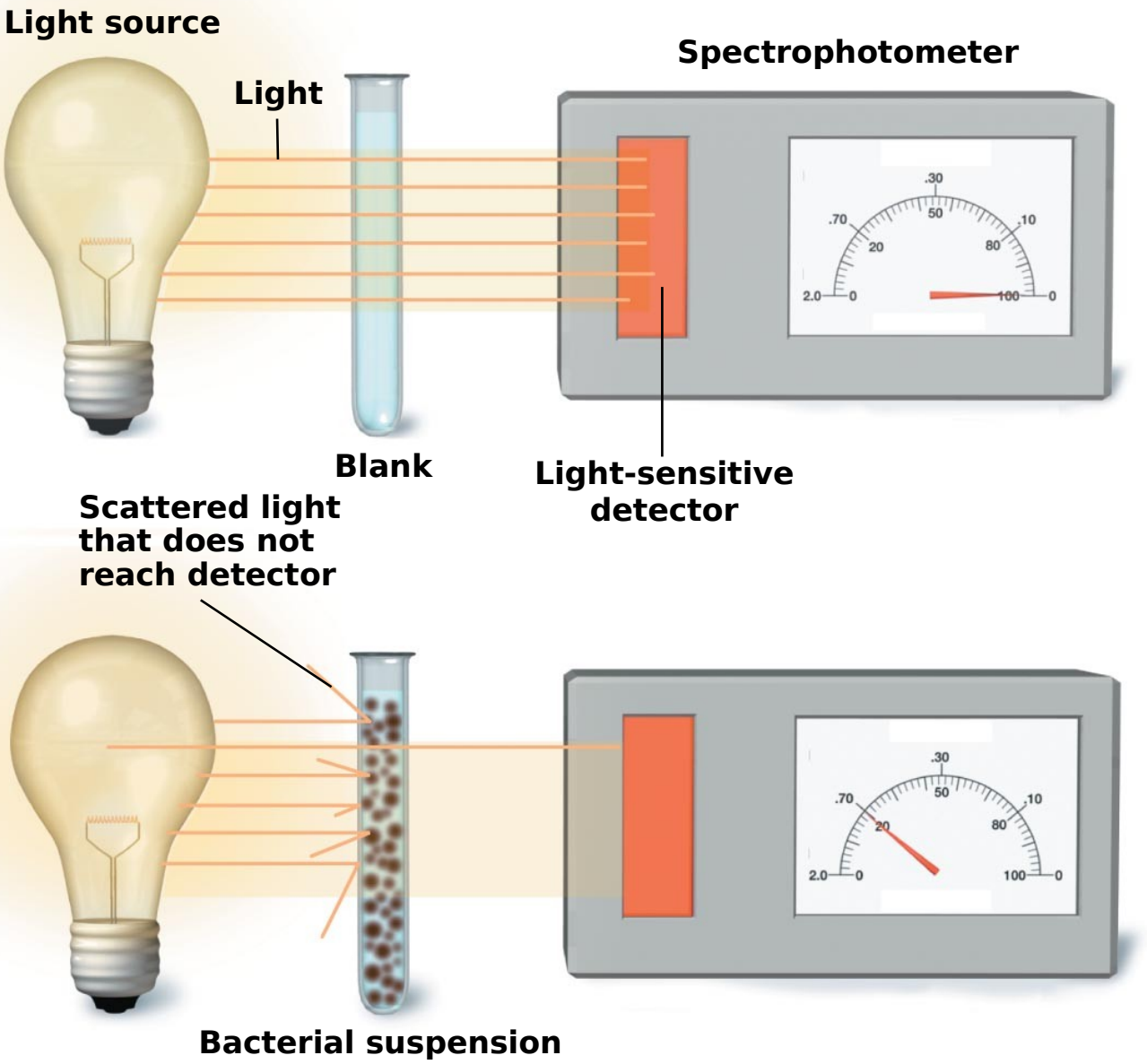
Check Your Understanding

- Why is it difficult to measure realistically the growth of a filamentous mold isolate by the plate count method?
  6-16

Estimating Bacterial Numbers by Indirect Methods

- **Turbidity**—measurement of cloudiness with a spectrophotometer
- Metabolic activity—amount of metabolic product is proportional to the number of bacteria
- Dry weight—bacteria are filtered, dried, and weighed; used for filamentous organisms

Figure 6.21 Turbidity estimation of bacterial numbers.



Check Your Understanding

- ✓ Direct methods usually require an incubation time for a colony. Why is this not always feasible for analyzing foods?
  6-17
- ✓ If there is no good method for analyzing a product for its vitamin content, what is a feasible method of determining the vitamin content?
  6-18